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| **Official Title:** | **A Phase 2, Open Label, Randomized, Dose Ranging, Safety, Efficacy, Pharmacokinetic and Pharmacodynamic Study of AG-348 in Adult Patients with Pyruvate Kinase Deficiency** |
| **NCT Number:** | NCT02476916 |
| **Document Dates:** | Protocol Version 6: 30 June 2017<br>Protocol Version 5: 30 March 2016<br>Protocol Version 4: 10 November 2015<br>Protocol Version 3: 05 August 2015<br>Protocol Version 2: 02 February 2015<br>Protocol Version 1: 05 January 2015 |

# CLINICAL STUDY PROTOCOL AG348-C-003

## EudraCT No. 2015-000484-13

## A Phase 2, Open Label, Randomized, Dose Ranging, Safety, Efficacy, Pharmacokinetic and Pharmacodynamic Study of AG-348 in Adult Patients with Pyruvate Kinase Deficiency

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| Current Protocol Version (Date): | Amendment 5, Version 6.0 (30 June 2017), Final |
| Previous Protocol Versions (Dates): | Version 1.0 (05 January 2015), Final<br>Amendment 1, Version 2.0 (02 February 2015), Final<br>Amendment 2, Version 3.0 (05 August 2015), Final<br>Amendment 3, Version 4.0 (10 November 2015), Final<br>Amendment 4, Version 5.0 (30 March 2016), Final |

This study will be conducted according to the protocol and in compliance with Good Clinical Practice, the ethical principles stated in the Declaration of Helsinki, and other applicable regulatory requirements.

**CONFIDENTIALITY NOTE:**

The information contained in this document is confidential and proprietary to Agios Pharmaceuticals, Inc. Any distribution, copying, or disclosure is strictly prohibited unless such disclosure is required by federal regulations or state law. Persons to whom the information is disclosed must know that it is confidential and that it may not be further disclosed by them.

# SPONSOR PROTOCOL APPROVAL

I hereby approve this clinical study protocol on behalf of Agios Pharmaceuticals, Inc. and attest that it complies with all applicable regulations and guidelines.

**Approved by:**

[redacted], MD, PhD
[redacted]
Agios Pharmaceuticals, Inc.

[redacted]
Signature

30 June 2017
Date (DD MMM YYYY)

# INVESTIGATOR'S AGREEMENT

I understand that all documentation provided to me by Agios Pharmaceuticals, Inc. (Agios) or its designated representative(s) concerning this study that has not been published previously will be kept in strict confidence. This documentation includes the study protocol, the AG-348 Investigator's Brochure, case report forms, and other scientific data.

This study will not commence without the prior written approval of a properly constituted Institutional Review Board (IRB). No changes will be made to the study protocol without the prior written approval of Agios and the IRB, except where necessary to eliminate an immediate hazard to the subject.

I have read, understood, and agree to conduct this study as outlined in the protocol and in accordance with the guidelines and all applicable government regulations.

_____

Investigator Name (Printed)

_____ _____

Investigator Signature Date (DD MMM YYYY)

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Investigational site or name of institution and location (printed)

# 2. SYNOPSIS

**Name of Sponsor/Company:**
Agios Pharmaceuticals, Inc.

**Name of Investigational Product:**
AG-348

**Title of Study:**
A Phase 2, Open Label, Randomized, Dose Ranging, Safety, Efficacy, Pharmacokinetic and Pharmacodynamic Study of AG-348 in Adult Patients with Pyruvate Kinase Deficiency

**Study Center(s):**
This study will be conducted at multiple study centers.

**Phase of Development:** 2

**Objectives:**

**Core Period**

**Primary Objective:**

- Evaluate the safety and tolerability of up to 24 weeks of AG-348 administration in subjects with pyruvate kinase deficiency (PK deficiency).

**Secondary Objectives:**

- Evaluate the pharmacokinetics (PK) of AG-348 and the metabolite AGI-8702.
- Evaluate the pharmacodynamic (PD) response of adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG) after administration of AG-348.
- Evaluate indicators of clinical activity of AG-348 in subjects with PK deficiency, including changes in hemoglobin (Hb), hematocrit (HCT), reticulocyte count, haptoglobin (Hp), carboxyhemoglobin (COHb), lactate dehydrogenase (LDH), total and indirect bilirubin, erythropoietin (EPO), hepcidin, ferritin, and transferrin saturation (serum iron/ iron-binding capacity).

[redacted]

**Extension Period**

**Primary Objective:**

- Evaluate the safety and tolerability of up to 30 months of AG-348 administration in subjects with PK deficiency.

**Secondary Objectives:**

- Evaluate the PK of AG-348 and the metabolite AGI-8702.
- Evaluate the PD response of ATP and 2,3-DPG after administration of AG-348.
- Evaluate indicators of clinical activity of AG-348 in subjects with PK deficiency, including changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, hepcidin, ferritin, and transferrin saturation (serum iron/iron-binding capacity).
- Evaluate the optimal maintenance dose of AG-348 for each individual subject during the Extension Period.

[redacted]

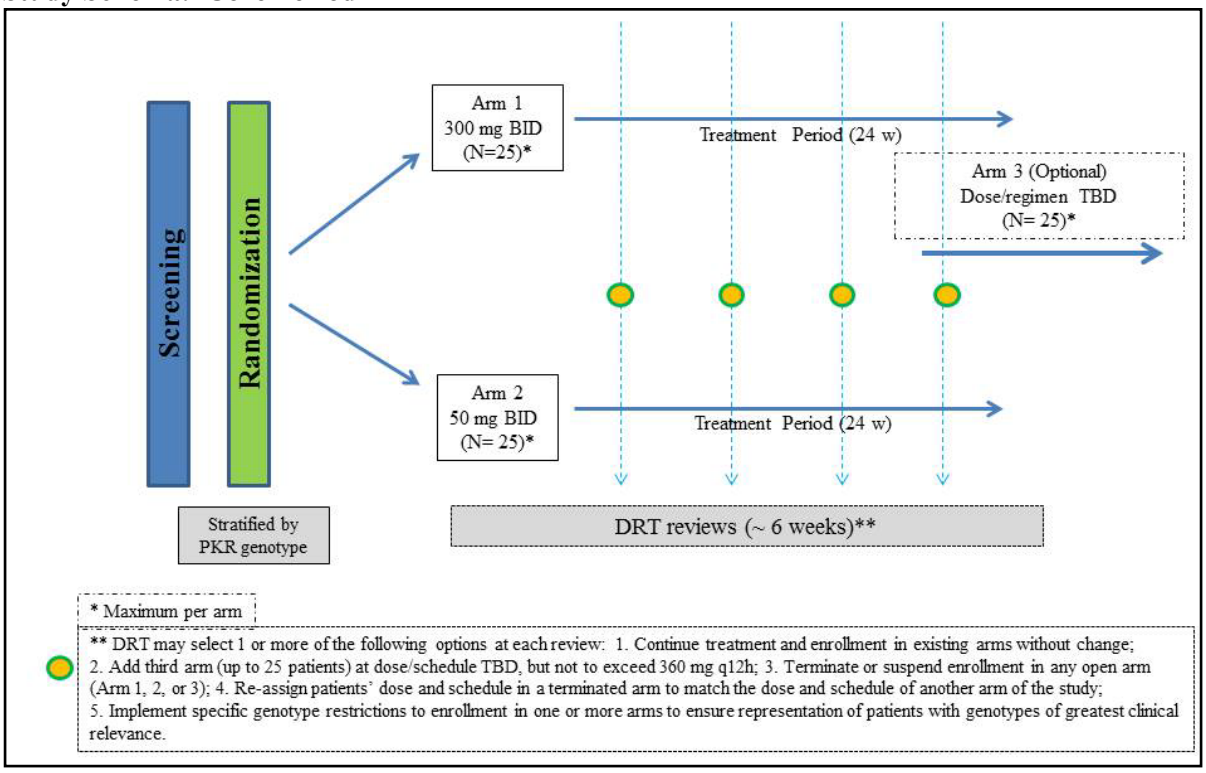
**Methodology:**
Study AG348-C-003 is a Phase 2, open label, 2-arm, multicenter, randomized, dose-ranging study in adult subjects with PK deficiency; the study will be divided in to a Core Period and an Extension Period. During the Core Period, subjects will receive multiple doses of AG-348 for up to 24 weeks; subjects who are eligible can enter the Extension Period to receive AG-348 for up to 2 years following the end of the Core Period.

Subjects with PK deficiency confirmed by red blood cell (RBC) PK enzymatic assay performed at Screening will be eligible to participate in this study. At the Week 24 visit, subjects who safely tolerate AG-348 and demonstrate clinical activity of AG-348 may be eligible to immediately roll over to the Extension Period for continued treatment. Subjects who complete treatment at the end of the Core Period (24 weeks) will undergo follow-up assessment 4 weeks after the last dose of study drug.

If a subject discontinues at any other time (including discontinuation during the Core or Extension Period), the follow-up assessments will be conducted 4 weeks after discontinuation. Subjects with toxicity suspected to be related to study drug will continue follow-up until the adverse event (AE) resolves, is declared chronic by the Investigator, or the subject is lost to follow-up.

For the Core Period, up to 25 subjects will be initially randomized on an open-label 1:1 basis to each of two twice-daily (BID) doses of AG-348 (up to 50 subjects total; refer to the Study Schema diagram below).

**Study Schema: Core Period**




Abbreviations: BID (q12h) = twice daily (every 12 hours); DRT = Data Review Team; PKR = pyruvate kinase red blood cell isoform; TBD = to be determined; w = week(s).

The dose of Arm 1 is 300 mg of AG-348 administered orally (PO) every 12 hours (q12h), ie, BID. The dose of Arm 2 is 50 mg of AG-348 administered PO BID.

Randomization will be stratified by PKR mutation in order to maintain balance as much as possible across the dose arms for the specific mutations expected to be most frequently enrolled. The PKR

mutation stratification factor will consist of 4 levels (R510Q, R486W, R479H, and all other mutations as "other"). Mutation status is defined by the presence of at least 1 of the indicated mutations; subjects with more than 1 stratified mutation will be assigned based on the Sponsor's discretion.

The doses for each arm of the Core Period have been selected from the AG348-C-001 single ascending dose (SAD) and AG348-C-002 multiple ascending dose (MAD) studies in healthy adult subjects to represent the range of doses/exposures that were safely tolerated and resulted in maximal or near-maximal PD effects on 2,3-DPG and ATP.

Because PK deficiency is a rare disease with a limited eligible subject population and because the underlying pathophysiology and clinical phenotype of affected subjects is heterogeneous due to the wide variety of mutations in PKR that cause the disease, it is deemed important to focus closely on dose findings in this first-in-patient study. Therefore, in addition to initiating this study with 2 different doses of AG-348 administered BID, a Data Review Team (DRT) will be established to review study data on a frequent basis and adapt the study design, dose, and schedule of AG-348 as indicated.

The DRT will monitor safety on an ongoing basis and meet at regular intervals of approximately every 6 weeks, or ad hoc, as necessary, for as long as any subjects are still in the Core Period to review AEs, vital signs (VS), clinical laboratory assessments (hematology, clinical chemistry, coagulation, and urinalysis), and electrocardiograms (ECGs). The DRT will also review available PK/PD data and indicators of clinical activity (eg, changes from baseline in Hb).

These DRT meetings will also include data review for all subjects that may be under treatment in the Extension Period. If there are no subjects still being treated in the Core Period, and the only subjects on treatment are those in the Extension Period, then the frequency of the DRT meetings will reduce to approximately every 4 to 6 months in order to match the frequency of subject visits (and new data collection) in the Extension Period.

When all the subjects are in the Extension Period, PK/PD data will no longer be reviewed by the DRT. The DRT will be comprised of the study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer.

Beginning 6 weeks after the first subject is dosed in the Core Period or ad hoc as necessary, and proceeding according to the schedule indicated above (approximately every 6 weeks during the Core Period, and approximately every 4 to 6 months during the Extension Period once all subjects have completed the Core Period), the DRT will review cumulative safety data, available PK/PD data, and clinical activity data.

Based on the DRT's recurring reviews, the DRT may exercise 1 or more of the following options during the Core Period:

- Continue treatment and enrollment in existing arms without change.
- Add 1 new dose arm (Arm 3) to enroll up to 25 subjects at a dose to be determined; the dose for Arm 3 may be lower or higher than Arm 1 and Arm 2 doses, but will not exceed 360 mg BID; and the dose regimen may be less frequent than BID.
- Terminate or suspend enrollment to allow further review of clinical data in Arm 1 and/or Arm 2 (and/or potential Arm 3). Enrollment in an arm could be terminated or suspended to allow further review, for example, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity.
- Re-assign subjects' doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the subjects in the terminated arm will remain in their original arm, ie, they will not count towards the enrollment quota of the arm whose dose and schedule is being adopted.

- Implement specific genotype restrictions to enrollment in 1 or more arms to ensure representation of subjects with genotypes of greatest clinical relevance.

The DRT may exercise 1 or more of the following options during the Extension Period:

- Continue treatment without change.
- Re-assign subjects' doses and schedule to an existing dose and schedule that has been determined to be safer, and/or have a better PD response, or produce signs of clinical activity.
- Terminate or suspend treatment to allow further review of clinical data (eg, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity).

The data that the DRT will review to make these decisions are expected to include, but are not necessarily limited to, the following:

- *Safety Observations:* All AEs, VS, clinical laboratory assessments (hematology, clinical chemistry, coagulation, and urinalysis), and ECGs.
- *PK and PD Observations:* Includes changes in 2,3-DPG and ATP, except when all subjects are in the Extension Period
- *Indicators of Clinical Activity:* Includes changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, EPO, hepcidin, total and indirect bilirubin, ferritin, and transferrin saturation.

If a third dose arm is implemented, the dose of AG-348 selected will not exceed 360 mg BID, as this was the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy adult subjects.

The PK/PD sampling schedule in a potential third dosing arm will be determined at the Sponsor's discretion and may follow either the extensive or limited PK/PD sampling schedules as specified.

The DRT will monitor the data in an ongoing manner as described and may make a decision to terminate enrollment in an arm based on evaluation of safety and efficacy.

All subjects in the Extension Period will undergo a gradual dose-taper regimen to identify the optimal maintenance dose for each (defined as the dose that results in ≤ 1.0 g/dL decrease in Hb compared to the pretaper Hb value on at least 2 measurements following each dose taper step) on a per-subject basis (see Section 9.7.3).

Due to the potential for AG-348-mediated aromatase inhibition, combined with the known risk of osteoporosis in subjects with congenital hemolytic anemias, dual-energy x-ray absorptiometry (DXA) scan (hip and spine) will be performed at Screening (if subject has not had prior DXA scan within 3 months of Day 1) to obtain bone mineral density values and T and Z scores. These data are intended to serve as a baseline measure of bone mineral density for all enrolling subjects, and are deemed of particular importance for those who enter the longer term Extension Period after completing 24 weeks of treatment (Core Period).

All subjects will have a second DXA scan in the interval between Weeks 24 and 28 for the Core Period. Subjects in the Extension Period will have additional DXA scans at Months 12, 18, 24, and 30.

As the number of enrolling arms changes in the study (for example, from 2 to 3), the randomization scheme will adjust to enable balanced randomization into each actively accruing arm. Randomization and stratification will cease in the event that only a single arm is left enrolling.
Depending on possible early termination of 1 or both of the initial 2 arms, or the addition of a third arm, the study could enroll up to a maximum of 75 subjects.

An extension study (to be determined) may be offered to subjects who successfully complete the Extension Period.

**Visit Schedule**

Screening assessments will occur within 42 days prior to the first dose of study treatment. During the Core Period, subjects will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12), and monthly through Week 24 (Weeks 16, 20, and 24).

Subjects who safely tolerate AG-348 through Week 24 (Core Period) and for whom the Investigator agrees with continuation of AG-348 treatment may be eligible to immediately enter the Extension Period for continued treatment for up to 2 years after completion of the Core Period, upon agreement of the treating Investigator and the Medical Monitor or Responsible Medical Officer.

Study visits for safety and clinical activity assessments will occur approximately every 3 months during the Extension Period, except during the dose taper part of the Extension Period when weekly visits will be performed. All subjects will undergo a follow-up assessment 4 weeks after the last dose of AG-348, regardless of whether this was due to discontinuation, the last dose in the Core Period for a subject who chooses not to continue in the Extension Period, or the last dose of the Extension Period (except for those who may continue treatment in an extension study [to be designed] after having completed the Extension Period).

**Dose Modifications for Safety and/or Increase in Hb Level**

The Investigator will monitor all subjects for safety and tolerability. Modification of an individual subject's dose of AG-348 will be based on AEs and/or observed changes in Hb level as described in Section 9.7.1 and Section 9.7.2.

Additional criteria for dose reduction will apply in the Extension Period, as detailed in Section 9.7.3.

**Number of subjects (planned):** Up to approximately 75 subjects.

**Diagnosis and main criteria for inclusion:**

**Inclusion Criteria**

***Core Period:***

Subjects must meet all of the following criteria to be eligible for inclusion in the Core Period of the study:

1. Have provided signed written informed consent prior to undergoing any study procedure, including Screening procedures.
2. Be male or female, aged 18 years and older.
3. Have a known medical history of PK deficiency.
4. Have documented clinical laboratory confirmation of PK deficiency by RBC pyruvate kinase enzymatic assay performed at Screening, either by a designated central laboratory or by any participating investigative site's local hematology laboratory. Subjects with prior documentation of PK deficiency by RBC enzymatic assay must have reconfirmation of this result during Screening as a condition of enrollment.

   NOTES:

   i. In the event that a subject's Screening pyruvate kinase enzymatic assay is negative (ie, shows normal pyruvate kinase activity), the subject will be eligible for enrollment if the genotyping shows a mutant genotype that has been previously documented in the literature to be associated with PK deficiency.

   ii. If the genotyping shows a previously undescribed mutation in the PKR gene, the subject's eligibility for enrollment will be determined on an individual case basis by the Coordinating Investigator and Medical Monitor in discussion with the Investigator.

   iii. If no mutation is defined, the subject will not be eligible for enrollment.
5. Have a blood sample for genotypic characterization of the mutant PKR gene performed by the designated central laboratory at Screening.

NOTES:

i. The designated central laboratory-determined genotype will generally serve as the basis for genotyping for enrollment. However, subjects whose genotype has already been determined by another laboratory may be enrolled on the basis of that report, with the approval of the Medical Monitor, in the case of an unexpected delay in return of the designated central laboratory result during the Screening Period.

ii. Enrollment on the basis of a result from a laboratory other than the designated central genotyping laboratory does not relieve the inclusion requirement that ALL subjects must have a sample sent to the designated central genotyping laboratory.

6. Have Hb ≤ 12.0 g/dL (if male) or ≤ 11.0 g/dL (if female).
7. Be considered transfusion-independent, defined as having had ≤ 3 units of RBCs transfused in the 12-month period up to the first day of study drug dosing and no transfusions within 4 months of the first day of study dosing.
   NOTE: Subjects who have received more transfusion support than described above will be evaluated for eligibility on a case-by-case basis by the Medical Monitor.
8. Have their spleen in place or have undergone splenectomy. Splenectomized subjects must meet all of the following conditions:
   i. Have undergone the procedure ≥ 6 months prior to Screening.
   ii. Be current in their vaccinations for pneumococcal conjugate (PCV13), pneumococcal polysaccharide (PPSV23), quadrivalent meningococcal vaccine, and *Haemophilus influenzae* Type B, as recommended by the United States Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices (refer to http://www.cdc.gov/vaccines/schedules/downloads/adult/adult-schedule.pdf) or, for subjects in Canada or the European Union, by immunization advisory groups in those locations.
   NOTE: Any missing vaccinations may be administered, starting with the Screening Period and continuing throughout the trial, following the initiation of AG-348 dosing and as necessary according to recommended vaccination guidance.
9. Have Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 2.
10. Have been taking ≥ 1 mg of folic acid daily for ≥ 21 days prior to the first dose of study drug and agree to continue this regimen during the study.
11. Have adequate organ function, defined as meeting all of the following conditions:
   i. Serum aspartate aminotransferase (AST) ≤ 2.5 × upper limit of normal (ULN), unless the increased AST is assessed by the Investigator as due to hemolysis and/or hepatic iron deposition, and alanine aminotransferase (ALT) ≤ 2.5 × ULN, unless the increased ALT is assessed by the Investigator as due to hepatic iron deposition.
   ii. Either normal or elevated levels of serum bilirubin. In subjects with serum bilirubin > ULN, the elevation must be attributed to hemolysis with or without Gilbert's syndrome and must not be attributed to choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease.
   iii. Serum creatinine ≤ 1.25 × ULN or, if > 1.25 × ULN, then 24-hour measured or calculated (by Cockcroft-Gault) glomerular filtration rate ≥ 60 mL/min.
   iv. Absolute neutrophil count (ANC) ≥ $1.0 \times 10^9$/L.
   v. Platelet count ≥ $100 \times 10^9$/L.
   vi. Activated partial thromboplastin time (aPTT) and international normalized ratio (INR) ≤ 1.25 × ULN, unless the subject is receiving therapeutic anticoagulants.
12. For women of childbearing potential—defined as females who either have experienced menarche and have not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, and/or bilateral oophorectomy) or who are not postmenopausal (defined as either having amenorrhea for ≥ 12 consecutive months without another cause and documented serum follicle-stimulating hormone [FSH] level > 35 mIU/mL or being ≥ 62 years of age and

having amenorrhea for ≥ 12 consecutive months [no FSH testing required]):

i. Agree to abstain from sexual intercourse or to use an acceptable/effective method of contraception (ie, condom plus spermicide, condom plus oral contraceptive, condom plus intrauterine device, condom plus diaphragm with spermicide) from as soon as is feasible during the Screening period until 30 days following the last dose of AG-348.
NOTE: Abstinence is an acceptable method only when this is in line with the normal lifestyle of the subject, meaning that the subject plans to remain abstinent *continuously* throughout the duration of the study and for ≥ 30 days after the last dose of study drug. Periodic abstinence (eg, calendar, symptothermal, and postovulation methods) and withdrawal are not acceptable methods of contraception.

ii. Have negative serum or urine pregnancy test within 72 hours before start of AG-348 dosing.

iii. Not be breastfeeding.

13. If male (with the exception of subjects who have undergone vasectomy ≥ 6 months prior to Screening), agree to abstain from sexual intercourse or, if sexually active, to use a condom with spermicide as contraception (regardless of their female partner's childbearing potential or their partner's use of their own contraception) from Day 1 of dosing until 30 days following the last dose of AG-348.
NOTE: Abstinence is an acceptable method only when this is in line with the normal life style of the subject, meaning that the subject plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence (eg, selective timing of intercourse based on partner's calendar, symptothermal, and postovulation methods) and withdrawal are not acceptable methods of contraception.

***Extension Period***

Subjects must meet all of the following criteria to be eligible for inclusion in the Extension Period of the study:

1. Have provided signed written informed consent prior to undergoing any study procedure during the Extension Period.
2. Have completed 24 weeks of treatment during the Core Period and tolerated AG-348 (defined as having completed 24 weeks with or without protocol-permitted dose modifications).
3. The treating Investigator agrees that there is a potential for clinical benefit to the subject from continued treatment and recommends participation in the Extension Period.
4. The Sponsor's designated Medical Monitor or Responsible Medical Officer approves the subject's participation in the Extension Period.
5. If applicable, agree to continue to follow the same sexual abstinence/contraception rules as stated above for the Core Period (Inclusion Criterion 12 [for females] or 13 [for males]).

**Exclusion criteria:**

***Core Period***

Subjects who meet any of the following criteria will be excluded from the Core Period of the study:

1. Have Hb level > 12.0 g/dL (if male) or > 11.0 g/dL (if female).
2. Have an additional diagnosis of any other congenital or acquired blood disorder, including glucose-6-phosphate-dehydrogenase (G6PD) deficiency, or any other hemolytic anemia process, with the exception of mild allo-immunization as a consequence of transfusion therapy.
3. Have iron overload (hemosiderosis or concurrent hemochromatosis) sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac, hepatic, or pancreatic insufficiency.
4. Have had prior bone marrow or stem cell transplant.
5. Have clinically symptomatic cholelithiasis or cholecystitis.

NOTES:

i. Prior cholecystectomy is not exclusionary.
ii. Subjects with symptomatic cholelithiasis or cholecystitis may be re-screened once the disorder has been treated and clinical symptoms have resolved.

6. Be currently enrolled in another therapeutic clinical trial involving ongoing therapy with any investigational or marketed product or placebo.
   NOTE: Concurrent participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480) is permitted.
7. Have been exposed to any investigational drug, device, or procedure within 28 days prior to Screening or during trial participation
8. Have any concurrent medical condition that could compromise participation in the study, such as:
   i. Poorly controlled hypertension (defined as systolic blood pressure [BP] > 150 mm Hg or diastolic BP > 90 mm Hg) refractory to medical management.
   ii. History of recent (within < 6 months from Screening date) congestive heart failure; myocardial infarction or unstable angina pectoris; hemorrhagic, embolic, or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.
   iii. Currently active infection requiring the use of parenteral antimicrobial agents or of ≥ Grade 3 severity (per National Cancer Institute Common Terminology Criteria for Adverse Events [CTCAE] v4.03) within 6 months of first dose of study drug.
   iv. Pattern or frequency of postsplenectomy sepsis that, in the assessment of the Investigator, could reasonably be expected to interfere with the ability of the subject to complete participation in the 24-week Core Period of the study.
   v. Positive test for hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV) antibody with accompanying signs of active hepatitis B or C infection.
   vi. Positive test for human immunodeficiency virus (HIV) 1 or 2 antibody.
   vii. Diabetes mellitus that is judged to be in poor control by the Investigator or which requires > 3 anti-diabetic agents, counting insulin (all insulins are considered 1 agent).
   NOTE: Use of insulin per se is not exclusionary.
   viii. History of any primary malignancy, with the exception of: curatively treated nonmelanomatous skin cancer, curatively treated cervical or breast carcinoma in situ, or any other primary tumor treated with curative intent, and with no known active disease present and no anticancer treatment administered during the last 3 years.
9. Have undergone major surgery within 6 months of first dose of study drug.
10. Have currently or have a recent history of a psychiatric disorder that, in the opinion of the Investigator or Medical Monitor, could compromise the ability of the subject to cooperate with study visits and procedures.
11. Have used any of the restricted list of products known to strongly inhibit cytochrome P450 (CYP) 3A4 drug metabolism (Appendix 4.1) within 5 days prior to Day 1 dosing; products known to strongly induce CYP3A4 metabolism (Appendix 4.2) within 28 days prior to Day 1 dosing; products known to strongly inhibit P-glycoprotein (P-gp) transporter (Appendix 4.3) within 5 days prior to Day 1 dosing; or digoxin within 5 days prior to Day 1 dosing.
12. Have serum bilirubin > ULN attributable to factors other than hemolysis and/or Gilbert's syndrome.
13. Have heart-rate corrected QT interval by Fridericia's method (QTcF) > 450 msec (for males) or > 470 msec (for females), with the exception of subjects with a left bundle branch block, for whom Medical Monitor approval is needed to enroll.
14. Have cardiac dysrhythmia that is judged clinically significant by the Investigator or which requires therapy with drugs that are primarily substrates of CYP3A4.

15. Have any history of allergy to sulfonamides characterized by acute hemolytic anemia, anaphylaxis, or rash of erythema multiforme type or Stevens-Johnson syndrome.
16. Have any other medical or psychological condition regarded by the Investigator as likely to interfere with subject's ability to understand and provide signed written informed consent; cooperate with study visits, tests, and procedures; and/or otherwise safely and reliably participate in the study.

***Extension Period:***
Subjects who meet this criterion will be excluded from the Extension Period of the study:

1. Have experienced any AE during the Core Period considered by the treating Investigator or the Sponsor's designated Medical Monitor or Responsible Medical Officer to pose a significant safety risk should study treatment be extended.

**Investigational product, dosage, and mode of administration:**

AG-348 sulfate hydrate capsules will be provided as 5 mg, 25 mg, or 100 mg (free-base equivalent) of AG-348 sulfate hydrate without excipients in hard gelatin capsules.

AG-348 will be administered PO BID. The number of capsules per dose will vary by assigned dose group.

AG-348 will be administered with water and may be administered with or without food.

**Reference therapy, dosage and mode of administration:**
Not applicable.

**Duration of treatment:**
The duration of treatment for all subjects in the Core Period will be up to 24 weeks. Subjects who safely tolerate AG-348 and for whom the Investigator agrees with continuation of AG-348 treatment may be eligible to immediately roll over to the Extension Period for continued treatment (up to 2 years following completion of the Core Period).

**Criteria for evaluation:**

**Safety**
Monitoring of AEs in randomized subjects, including serious AEs (SAEs), AEs of special interest (AESIs), and AEs leading to discontinuation (all AEs will be graded using CTCAE, Version 4.03); safety laboratory parameters (hematology, chemistry, urinalysis, coagulation); physical examination findings (including neurological examination); VS; 12-lead ECGs; and DXA scans.

Serum sex hormone levels (testosterone [total and free], estrone, and estradiol), bone turnover markers (serum osteocalcin-N-mid and serum C-terminal telopeptide [CTX]), 25-hydroxy vitamin D2 and D3, total cholesterol, high-density lipoprotein-cholesterol (HDL-C), and triglycerides will be monitored for evidence of potential inhibition of aromatase by AG-348.

Menstruating female subjects will also keep a paper-based menstrual cycle diary throughout the study.

**Indicators of Clinical Activity**

Monitoring of potential indicators of clinical activity will include evaluating changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, hepcidin, ferritin, and transferrin saturation.

**Pharmacokinetics**
Approximately the first 10 subjects treated in the Core Period, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 1.3. The remainder of treated subjects will undergo limited PK sampling as detailed in Appendix 1.4.

During the Core Period, serial blood sampling for determination of concentration-time profiles of AG-348 and its metabolite AGI-8702 will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of AG-348 and AGI-8702 will be obtained.

During the Extension Period, predose PK samples will be drawn for the measurement of trough levels of AG-348 and AGI-8702 at each study visit t (every 3 months; Appendix 1.2). AG-348 and AGI-8702 will be analyzed using qualified assays to determine concentrations in plasma.

**Pharmacodynamics**

Pharmacodynamic assessments during the Core Period will include 2,3-DPG, ATP (secondary objectives), [redacted] Approximately the first 10 subjects treated during the Core Period will undergo extensive PD sampling as detailed in Appendix 1.3. The remainder of treated subjects will undergo limited PD sampling as detailed in Appendix 1.4. During the Core Period, serial blood sampling for determination of levels of ATP and, 2,3-DPG will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of ATP and 2,3-DPG will be obtained.

During the Extension Period, predose PD samples will be drawn for the measurement of trough levels of 2,3-DPG, ATP, [redacted] at each study visit (every 3 months; Appendix 1.2). Adenosine triphosphate and 2,3 DPG will be analyzed using qualified assays to determine concentrations in whole blood. Pharmacodynamic parameters on Day 1 and Day 15 will be computed based on observed whole blood ATP and 2,3-DPG concentrations.

[redacted]

**Statistical methods:**

**Sample Size**

Due to the rare disease setting, the minimal sample size may be determined by feasibility. Depending on possible early termination of 1 or both of the initial 2 arms or the addition of a 3rd dose arm, the study could enroll up to a maximum of 75 subjects.

**Analysis Periods**

Analyses of safety and of indicators of clinical activity will be separated for the Core Period, and for the Cumulative Period (Core Period and Extension Period), if applicable. Unless specified otherwise, safety analysis will be based on the treatment-emergent period defined as from the first dose to 30 days after the last dose of the corresponding period. Efficacy analysis will be based on the efficacy window defined as from the first dose to 1 day after the last dose of the corresponding period.

**Analysis Sets**

Populations for analysis (ie, analysis sets) will include a Safety Analysis Set and an Efficacy Analysis Set. The Safety Analysis Set will include all subjects who receive at least 1 dose of study treatment; this population will be the primary set for the analysis of safety data and the default analysis set for all data analyses unless otherwise stated. Subjects will be classified according to initial treatment group, defined as the assigned treatment if it is received at least once, or as the first treatment received if assigned treatment is never received.

The Efficacy Analysis Set will include all subjects who are enrolled and received any study treatment for at least 3 weeks. The Efficacy Analysis Set will be the primary set for the analysis of preliminary clinical activity data. Subjects will be classified according to assigned treatment, unless specified otherwise.

**Statistical Analysis**

This section presents a summary of the planned statistical analyses of efficacy and safety for this study.

Statistical analysis and presentation details will be provided in the Statistical Analysis Plan (SAP) for the study, which will be finalized before the database lock after all subjects have completed the Core Period. The results of this analysis will be presented in a clinical study report (CSR).

Additional data collected during the Extension Period after the Core Period database lock will be analyzed for inclusion in a subsequent CSR addendum.

The primary objective during the Core Period of this Phase 2 study is to evaluate the safety and tolerability of up to 24 weeks of AG-348 in subjects with PK deficiency. Therefore, analyses will be primarily descriptive in nature; no formal hypothesis testing will be conducted.

Summaries will be produced for disposition, baseline disease characteristics and demographic data.

Categorical variables will be summarized by frequency distributions (number and percentages) and continuous variables will be summarized by descriptive statistics (mean, standard deviation [SD], median, minimum, and maximum).

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), and the incidence of AEs in the treatment-emergent period (TEAEs) (new or worsening from baseline) will be summarized by primary MedDRA System Organ Class (SOC) and Preferred Term, severity, outcome, action taken with study drug, and relationship to study drug.

Separate summaries will be produced for all TEAEs, treatment-related AEs (ie, those considered by the Investigator as at least possibly study drug-related), SAEs, AESIs, AEs leading to treatment discontinuation, and AEs ≥ Grade 3 severity. Individual subject listings will be provided for any deaths, SAEs, and TEAEs leading to treatment modification, interruption, or discontinuation.

Descriptive statistics will be provided for clinical laboratory values (eg, hematology, serum chemistry, coagulation studies, urinalysis) and VS, presented as both actual values and changes from baseline relative to each on-study evaluation. Shift analyses will be conducted for selected laboratory parameters based on the baseline CTCAE grade to maximum CTCAE grade. Where applicable CTCAE terms do not exist, a grading system based on the upper and/or lower limits of normal will be used to classify the results.

Electrocardiogram analyses will include individual subject listings and summaries of abnormal and clinically significant ECG results. Actual values and changes from baseline in the portion of the ECG wave from the beginning of the P wave to the beginning of the QRS complex (PR), QRS, heart rate-corrected QT intervals (QTc) will be summarized by visit.

Data collected from the menstrual diaries such as the start and stop dates of the menses and the subject reported characteristics of the menses will be presented in a by-subject listing. Additional descriptions of the data may also be performed.

Concomitant medications will be coded using the World Health Organization Drug Dictionary. Concomitant medications prior to and after the start of the study drug will be listed by subject, and summarized by summarized by Anatomical Therapeutic Chemical Classification System term and dose arm.

Hormone data, including the actual values and their changes from baseline at each visit will be summarized by sex using descriptive statistics (mean, SD, median, min and max). Spaghetti plots will be provided by sex.

Details of analyses to evaluate indicators of potential clinical activity of AG-348 in subjects with PK deficiency will be described in the SAP. These will include changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, hepcidin, ferritin, and transferrin saturation (serum iron/iron-binding capacity).

**Analysis Related to Dose-Tapering Regimen in the Extension Period**

The number and percentage of subjects at the individual optimal maintenance dose will be summarized. If necessary, additional analyses related to the dose-tapering regimen in the Extension Period, focusing on the long-term treatment effect in the Cumulative Period, may be conducted. These analyses will be described in a separate analysis plan for the Extension Period following the Core Period database lock, but before the database lock for the Extension Period.

**PK-PD Analysis**

Descriptive statistics will be used to summarize PK parameters for the parent compound AG-348 and the metabolite AGI-8702 for each dose group, and where appropriate, for the entire population. Pharmacokinetic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation.

Descriptive statistics will be used to summarize PD parameters for 2,3-DPG and ATP for each dose group, and where appropriate for the entire population. Pharmacodynamic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation %. Additional analyses, if conducted, may be described in a separate analysis plan.

**Interim Review**

No formal statistical interim analysis will be conducted.

Safety data will be reviewed on an ongoing basis by the DRT, who will meet to review safety, PK, PD, and preliminary clinical activity data at regular intervals (approximately every 6 weeks during the Core Period and approximately every 4 to 6 months during the Extension Period once all subjects have completed the Core Period) throughout the duration of the study. When all the subjects are in the Extension Period, PK/PD data will no longer be reviewed by the DRT. The composition of the DRT, its meeting schedule, the data to be reviewed, and the decisions it is empowered to make have been described previously.

Additional interim reviews of data may be conducted to support decision-making concerning the current clinical study, the Sponsor's development programs in general, or in case of any safety concerns.

# 3. TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

## TABLE OF CONTENTS

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# 4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

| Abbreviation or Specialist Term | Definition |
|---|---|
| 2,3-DPG | 2,3-diphosphoglycerate |
| ADP | Adenosine diphosphate |
| AE | Adverse event |
| AESI | Adverse event of special interest |
| ALC | Absolute lymphocyte count |
| ALT | Alanine aminotransferase |
| ANC | Absolute neutrophil count |
| aPTT | Activated partial thromboplastin time |
| AST | Aspartate aminotransferase |
| ATP | Adenosine triphosphate |
| AUC | Area under the curve |
| $AUC_{0\text{-}12hr}$ | Area under the plasma concentration versus time curve from 0 to 12 hours |
| $AUC_{0\ 24hr}$ | Area under the plasma concentration versus time curve from 0 to 24 hours |
| $AUC_{0\text{-}\infty}$ | Area under the plasma concentration versus time curve from 0 to infinity |
| BCRP | Breast cancer resistance protein |
| BID | Twice daily |
| BP | Blood pressure |
| BUN | Blood urea nitrogen |
| CBC | Complete blood count |
| $CO_2$ | Carbon dioxide |
| COHb | Carboxyhemoglobin |
| CSR | Clinical study report |
| CTCAE | Common Terminology Criteria for Adverse Events |
| CTX | Serum C-terminal telopeptide |
| CV | Cardiovascular |
| DDI | Drug-drug interaction |
| CYP | Cytochrome P450 |
| DLT | Dose-limiting toxicity |
| DRT | Data review team |

| Abbreviation or Specialist Term | Definition |
|---|---|
| DXA | Dual-energy x-ray absorptiometry |
| ECG | Electrocardiogram |
| ECOG | Eastern Cooperative Oncology Group |
| eCRF | Electronic case report form |
| EDC | Electronic data capture |
| EPO | Erythropoietin |
| FSH | Follicle-stimulating hormone |
| G6PD | Glucose-6-phosphate-dehydrogenase |
| GCP | Good Clinical Practice |
| GLP | Good Laboratory Practice |
| Hb | Hemoglobin |
| HBsAg | Hepatitis B surface antigen |
| HCT | Hematocrit |
| HCV | Hepatitis C virus |
| HDL-C | High-density lipoprotein-C |
| HIV | Human immunodeficiency virus |
| Hp | Haptoglobin |
| $IC_{50}$ | Concentration of drug that achieved half-maximal inhibition |
| ICH | International Conference on Harmonisation |
| IEC | Independent Ethics Committee |
| INR | International normalized ratio |
| IRB | Institutional Review Board |
| LDH | Lactate dehydrogenase |
| MAD | Multiple ascending dose |
| MCH | Mean corpuscular hemoglobin |
| MCHC | Mean corpuscular hemoglobin concentration |
| MCV | Mean corpuscular volume |
| mPKR | Pyruvate kinase isoform R mutants |
| NCI | National Cancer Institute |
| NOAEL | No-observed-adverse-effect-level |
| P-gp | P-glycoprotein |
| PCV13 | Pneumococcal conjugate |

| Abbreviation or Specialist Term | Definition |
|---|---|
| PD | Pharmacodynamic |
| PEP | Phosphoenolpyruvate |
| PK | Pharmacokinetic |
| PK deficiency | Pyruvate kinase deficiency |
| PKM2 | Pyruvate kinase isoform M2 |
| PKR | Pyruvate kinase isoform R |
| PO | Oral(ly) |
| PPSV23 | Pneumococcal polysaccharide |
| PR | The portion of the ECG wave from the beginning of the P wave to the beginning of the QRS complex |
| q12h | Every 12 hours |
| QD | Once daily |
| QTc | Heart-rate corrected QT interval |
| QTcB | Corrected QT interval – Bazett's correction formula |
| QTcF | Corrected QT interval – Fridericia's method |
| RBC | Red blood cell (count) |
| RDW | Red cell distribution width |
| SAD | Single ascending dose |
| SAE | Serious adverse event |
| SAP | Statistical analysis plan |
| SD | Standard deviation |
| $t_{½}$ | Apparent terminal half-life |
| TIBC | Total iron-binding capacity |
| $t_{max}$ | Time to maximum plasma concentration |
| TEAE | Treatment-emergent adverse event |
| ULN | Upper limit of normal |
| VS | Vital signs |
| WBC | White blood cell |
| WMA | World Medical Association |
| WT | Wild-type |

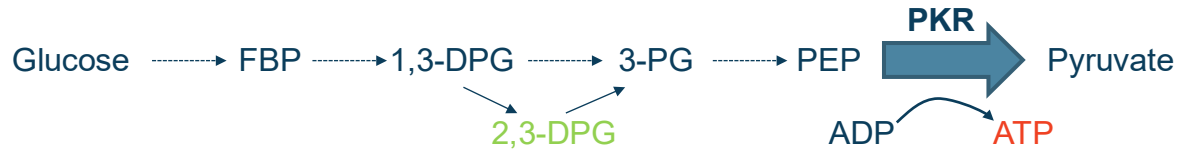
# 5. INTRODUCTION

## 5.1. Pyruvate Kinase Deficiency

Pyruvate kinase deficiency (PK deficiency) is a glycolytic enzymopathy that results in nonspherocytic hemolytic anemia with a variable clinical presentation, ranging from mild or fully compensated forms to life-threatening neonatal anemia and lifelong chronic hemolytic anemia associated with severe, debilitating comorbidities. Pyruvate kinase deficiency is caused by mutations in the PKLR gene, which results in defective pyruvate kinase isoform R (PKR) in the red blood cell (RBC).

As shown in Figure 1 below, PKR catalyzes the final and irreversible step in glycolysis, converting phosphoenolpyruvate (PEP) to pyruvate, and formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP). Mature RBCs rely almost exclusively on the process of glycolysis to generate the energy carrier molecule ATP. PKR is thus a key enzyme for maintaining energy homeostasis in erythrocytes, and it has been proposed that ATP levels are critical for optimally maintaining RBC membrane integrity (van Wijk and van Solinge 2005).

**Figure 1: Red Cell Glycolysis**




Abbreviations: 1,3 DPG = 1,3 diphosphoglycerate; 2,3 DPG = 2,3 diphosphoglycerate; 3-PG = 3-phosphoglycerate; ADP = adenosine diphosphate; ATP = adenosine triphosphate; FBP = fructose 1,6-bisphosphate; PEP = phosphoenolpyruvate; PKR = red blood cell-specific form of pyruvate kinase.
Note: Not all steps in glycolysis are shown.

The majority of mutations in PKR that have been described have a deleterious effect on PKR catalytic activity, protein stability, and/or protein expression. Pyruvate kinase deficiency is associated with reduced RBC survival as well as impaired red cell maturation. Mature erythrocytes lack mitochondria relying predominantly on glycolysis to generate ATP. It is hypothesized that insufficient energy production promotes erythrocyte hemolysis due to impaired maintenance of cellular membrane homeostasis.

Pyruvate kinase deficiency is an autosomal recessive disease, in which both homozygotes and compound heterozygotes develop hemolytic anemia. More than 200 different mutations in the PKLR gene have been identified in subjects with PK deficiency to date, and the majority of these mutations are single nucleotide missense mutations (Zanella, et al. 2005). The prevalence of PK deficiency, based on the most common pyruvate kinase mutation in Southern Europe, is estimated at 51 cases per million in the Caucasian population (Beutler and Gelbart 2000).

Some subjects with PK deficiency have a clinically severe disease course that can present as a severe hemolytic anemia in early infancy, requiring immediate care which typically includes blood transfusion support (Christensen, et al. 2011; Ghidini, et al. 1991; Hennekam, et al. 1990). Infants with PK deficiency may have erythroblastosis fetalis with hepatosplenomegaly, extramedullary hematopoiesis, and severe indirect hyperbilirubinemia, which is associated with a high risk of kernicterus.

Hyperbilirubinemia associated with the hemolytic state often requires supportive treatment with intensive phototherapy. For other infants, the hyperbilirubinemia caused by hemolysis can require exchange transfusion, as well as supportive transfusion to keep their hematocrit (HCT) levels > 60%.

Adults with PK deficiency are reported to have life-long hemolysis and the subsequent associated comorbidities including anemia and transfusion dependence. Other comorbidities include frequent miscarriages, aplastic crises, as well as symptoms associated with an acute or chronic hemolytic anemia (Rider, et al. 2011). Since unconjugated bilirubin is often chronically elevated, pigmented gallstones are common in children and adults. Severe and sometimes life-threatening iron deposition occurs and is typically progressive.

The current therapeutic options for subjects with PK deficiency are supportive. Most require life-long treatment, including blood transfusions at a frequency depending on the disease state. Long-term surveillance for systemic iron overload, even in transfusion-independent subjects, is standard as is the use of chelation therapy. Case reports of cure by allogeneic bone marrow transplant have been published (Tanphaichitr, et al. 2000) but have been infrequently performed. Splenectomy, while not curative, can reduce the need for supportive blood transfusions and decrease bilirubin levels in some subjects. However, this intervention is associated with significant morbidity including infection risk, and possible increased risk of thrombosis.

## 5.2. AG-348

AG-348 is an orally available, allosteric activator of PKR. It is hypothesized that drug intervention with AG-348 restores glycolytic pathway activity and normalizes red cell metabolism in vivo. Biochemical experiments demonstrate that AG-348 is a potent, broad-spectrum activator of many PKR alleles associated with PK deficiency. Pyruvate kinase-deficient red cells and their progenitors are characterized by changes in metabolism associated with defective glycolysis, including a build-up of PEP and 2,3-diphosphoglycerate (2,3-DPG), and lowered ATP levels. Ex vivo treatment of PK deficiency subject red cells with AG-348 results in increased ATP levels, and reductions in PEP and 2,3-DPG, consistent with pharmacological activation of PKR enzyme activity.

In vitro studies against wild-type (WT) PKR enzymes and ex vivo studies in blood from mice, monkeys and humans indicate that AG-348 activates WT PKR with a similar potency and efficacy as it does with the mutant versions. In vivo studies in mice confirm the potency of AG-348 in increasing WT PKR enzyme activity and modulating the levels of downstream markers such as ATP and 2,3-DPG. This therapeutic approach may be an effective way to correct the underlying pathology of PK deficiency and, importantly, provide clinical benefit to subjects.

### 5.2.1. Summary of Nonclinical Data

A series of exploratory pharmacology studies were conducted to characterize the ability of AG-348 to activate WT PKR and anemia-associated PKR mutants (mPKR) in vitro, ex vivo, and in vivo. Additional details regarding nonclinical data can be found in the AG-348 Investigator's Brochure.

#### 5.2.1.1. Pharmacology

AG-348 is a potent, broad-spectrum activator of PKR with low nM potency against both WT and mutant enzymes. AG-348 is also able to effectively stabilize mPKR enzymes that are hypersensitive to thermal denaturation, suggesting that AG-348 may be able to increase PKR pathway activity by preventing destabilization and degradation of mPKR enzymes. Its major metabolite, AGI-8702, has low potency mixed activity against PKR and mutant isoforms and can increase the thermostability of some mPKR, albeit with significantly weaker potency than that of AG-348.

The effect of AG-348 and AGI-8702 on PKR activity and a number of downstream pathway markers was evaluated in human RBCs. AG-348 dose-response curves in human whole blood showed potent activation of PKR, while AGI-8702 is a weak activator of the PKR enzyme. In whole blood from mice treated with AG-348, $^{13}$C-glucose labeling through the PKR reaction was increased by 80% demonstrating AG-348-mediated activation of the WT PKR enzyme. AG-348 treatment of RBCs from healthy human donors potently increased levels of the PKR reaction product ATP consistent with activation of PKR.

The effects of AG-348 on PKR activity and RBC metabolism also were assessed in blood samples from subjects with PK deficiency. AG-348 activated PKR and induced metabolic changes (increased ATP levels and decreased 2,3-DPG levels) consistent with increased glycolytic pathway activity in RBCs from PK deficiency subjects with different mutations in the PKR enzyme.

Assessments of binding inhibition potential and enzymatic activity were evaluated in a panel of 89 receptors, ion channels, and enzymes. Although AG-348 is a histamine H3 antagonist/inverse agonist and an aromatase inhibitor, no effects of histamine H3 modulation have been observed in safety pharmacology or toxicology studies. Effects consistent with aromatase inhibition were seen in toxicology studies (Section 5.2.1.4).

#### 5.2.1.2. Safety Pharmacology

Safety pharmacology studies demonstrated that neither AG-348 nor AGI-8702 exhibited significant inhibition of the human ether-à-go-go related gene (hERG) current (concentration of drug that achieved half-maximal inhibition [$IC_{50}$] 29.4 μM and > 10 μM, respectively). Additionally, in the monkey cardiovascular (CV) study, no CV effects were seen at any dose level. No effects were seen in AG-348 Good Laboratory Practice (GLP)-compliant Irwin and respiratory safety pharmacology studies in Sprague Dawley rats. AG-348 administered via oral gavage had a dose-dependent emetic activity in the ferret at ≥ 30 mg/kg.

#### 5.2.1.3. Pharmacokinetics

Absorption, distribution, metabolism, and excretion studies of AG-348 were performed in Sprague Dawley rats, beagle dogs, and cynomolgus monkeys. Both oral (PO) and intravenous (IV) routes were evaluated at doses ranging from 1 to 50 mg/kg. A dose of 200 mg (50 mg/kg) was administered PO in the food-effect evaluation in monkeys. The PK of AG-348 in animal species is characterized by rapid oral absorption, medium to high total body plasma clearance, and high volume of distribution at steady-state in rats, dogs, and monkeys.

AG-348 has a medium to long apparent terminal half-life ($t_{1/2}$) in the rat, dog, and monkey. Medium to high oral bioavailability was observed for AG-348 in animals. A high-fat diet led to lower oral absorption of AG-348 in monkeys.

AG-348 showed low brain penetration in rats (brain/plasma ratio of 5.7% to 9.1%) based on area under the plasma concentration versus time curve from 0 to 24 hours ($AUC_{0\text{-}24hr}$) following single and repeated-dose administration, respectively. Metabolism appears to be the major elimination pathway for AG-348 with minimal biliary or urinary excretion.

Both in vitro and in vivo, the predominant metabolite was the N-dealkylated product, AGI-8702. There were no metabolites unique to human liver microsomes and no direct Phase 2 metabolites. The plasma metabolite profile of AG-348 did not change with repeat or ascending dosing, suggesting that AG-348 is not susceptible to metabolic switching with increasing or prolonged dose administration.

AG-348 is a weak direct inhibitor of cytochrome P450 (CYP) 2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 (testosterone 6β-hydroxylation). There was no direct inhibition of CYP1A2, CYP2B6, or CYP3A4/5 (midazolam 1′-hydroxylation) by AG-348. There was evidence of metabolism-dependent inhibition of CYP2C19 (largely reversible) and CYP3A4 (largely irreversible) by AG-348. AG-348 is considered a weak time-dependent CYP3A4 inhibitor. AGI-8702 is a weak direct inhibitor of CYP2C9 and CYP2C19. AG-348 has the potential to cause an induction-related drug-drug interaction (DDI) with sensitive CYP2B6 and CYP3A4 substrates.

The routes of metabolism for AG-348 are via multiple CYPs with CYP3A4 contributing > 70% of the total. CYP1A2, CYP2C9, and CYP2C8 contribute approximately 6%, 10%, and 7% to the remaining metabolism of AG-348; other isoforms contribute < 4% each.

AG-348 is a substrate for P-glycoprotein (P-gp), but not breast cancer resistance protein (BCRP). AG-348 is an inhibitor of P-gp (91% and 99% inhibition at 41 and 411 μM, respectively), but does not inhibit BCRP. AGI-8702 is not an inhibitor of P-gp or BCRP under the concentrations tested (5 and 100 μM).

#### 5.2.1.4. Toxicology

The toxicity profile of AG-348 was evaluated in bacterial reverse mutation assays, the in vitro human peripheral blood micronucleus assay, the in vivo micronucleus assay, and in vivo in Sprague Dawley rats, New Zealand White rabbits, Beagle dogs, and cynomolgus monkeys. The toxicity of the major metabolite of AG-348, AGI-8702, was also evaluated through metabolic formation both in vitro in S9 fractions and in vivo.

AG-348 was nonmutagenic and nonclastogenic in GLP-compliant assays including bacterial reverse mutation assays, an in vitro micronucleus assay in human peripheral blood lymphocytes, and an in vivo micronucleus assay in male and female Sprague Dawley rats.

In dogs, following single oral dose administration, clinical observations consistent with anaphylactoid reactions were seen, and the maximum tolerated dose was 62.5 mg/kg, which was associated with an $AUC_{0\text{-}12hr}$ value of 8576 ng × h/mL. In monkeys, single oral doses of AG-348 up to 1000 mg/kg did not result in adverse findings, and were associated with an $AUC_{0\text{-}12hr}$ value of 105,000 ng × h/mL.

In the GLP-compliant 28-day monkey study, the high dose of 150 mg/kg/day (75 mg/kg/dose) was the no-observed-adverse-effect-level (NOAEL). Effects were limited to increased liver weights without serum chemistry or microscopic correlate.

In the 13-week repeat-dose study in monkeys, no adverse effects were identified at doses up to 200 mg/kg/day (100 mg/kg/dose; Day 90 $AUC_{0\text{-}12hr}$ values of 10,400 and 14,600 ng × h/mL in males and females, respectively), and no new effects were identified when compared to the 28-day repeat-dose study. In the 9-month repeat-dose monkey toxicology study, a previously unidentified adverse finding of minimal subcapsular inflammation/hepatocellular pressure necrosis resulting from nonadverse adaptive hepatocellular hypertrophy secondary to CYP enzyme induction was observed in the liver of males at 100 and 200 mg/kg/day (50 and 100 mg/kg/dose) and in females at 200 mg/kg/day (100 mg/kg/dose). No other adverse findings were observed.

In rats (Sprague Dawley), single oral doses of AG-348 up to 2000 mg/kg did not result in adverse findings, and were associated with $AUC_{0\text{-}12hr}$ values up to 789,327 ng × h/mL. In the GLP-compliant 28-day rat study, the NOAEL in males was the highest dose tested, 600 mg/kg/day (300 mg/kg/dose), and in females was the lowest dose tested, 20 mg/kg/day (10 mg/kg/dose).

In the 13-week repeat-dose rat study, adverse effects in males were identified in the testes and consisted of seminiferous tubular degeneration, spermatid retention, and Leydig cell hypertrophy. These effects were defined as adverse at dose levels at which they are expected to impair fertility. In the 13-week repeat-dose rat study, adverse effects in females were identified in the uterus and consisted of uterine atrophy and increased folding of the luminal surface. Similar to males, these effects were defined as adverse at the dose level at which they are expected to impair fertility. In the 6-month repeat-dose rat study, the highest dose tested was 300 mg/kg/day (150 mg/kg/dose) in males and 200 mg/kg/day (100 mg/kg/dose) in females, which resulted in no test article-related deaths.

AG-348 inhibits human aromatase activity with an $IC_{50}$ of 2050 nM (based on human placental microsomes) and rat aromatase with an $IC_{50}$ of 493 nM (based on rat ovarian microsomes). The potential adverse effects observed in the male and female reproductive tracts of rats in the 6-month toxicology study were consistent with aromatase inhibition and mirror findings in the previous 13-week toxicology study. AGI-8702 is not an aromatase inhibitor. In GLP-compliant embryo-fetal development studies in rats and rabbits, developmental toxicities were observed in rats but not rabbits. Fetal adverse effects in rats were considered likely due to aromatase inhibition.

For additional details of toxicology study findings, refer to the Investigator's Brochure.

### 5.2.2. Summary of Clinical Data

To date, 72 healthy adult subjects have been exposed to AG-348 in 2 clinical studies, a single ascending dose (SAD) study and a multiple ascending dose (MAD) study, with 31 of these subjects exposed to a single AG-348 dose under fasted conditions; 5 exposed to 2 doses of AG-348 under fasted and fed conditions; and 36 exposed to repeated doses of AG-348 for up to 14 days.

Except as otherwise specified, the following discussion of clinical data refers only to healthy adult subjects, as this is the first clinical trial in which subjects with PK deficiency will be treated with AG-348.

#### 5.2.2.1. Pharmacokinetics

The pharmacokinetics (PK) of AG-348 showed low to moderate variability between subjects. After single doses of AG-348 from 30 to 2500 mg, AG-348 was rapidly absorbed as indicated by the short time to maximum plasma concentration ($t_{max}$). There appeared to be some lengthening of the period of absorption at higher doses as indicated by higher $t_{max}$ and a less-than-proportional increase in maximum plasma concentration.

Dose-normalized area under the curve (AUC) generally remained constant over the dose range studied, suggesting that AG-348 total exposure increased in a dose-proportional manner. The mean $t_{1/2}$ ranged from 17.8 to 20.4 hours when samples were collected through 72 hours and from 50.3 to 79.3 when samples were collected through 120 hours. However, this terminal elimination phase contributed little to overall exposure of AG-348, as indicated by the small difference between $AUC_{0\text{-}12hr}$ and area under the plasma concentration versus time curve from 0 to infinity ($AUC_{0\text{-}\infty}$), suggesting a shorter effective half-life of approximately 3 to 6 hours.

AG-348 was extensively distributed (mean apparent volume of distribution range of 271 to 1148 L) and had a moderate rate of clearance (geometric mean clearance range of 10.3 to 13.9 L/hr). The fraction of AG-348 excreted in urine ranged from 0.0145 to 0.0209 across the dose levels, suggesting that renal excretion plays a minor role in the systemic elimination of AG-348.

The preliminary repeat-dose PK of AG-348 at doses ranging from 15 mg every 12 hours (q12h), ie, twice daily (BID), to 700 mg BID also showed an increase in AG-348 exposure in a dose-proportional manner after the first dose. AG-348 exposure observed after repeated dosing for 14 days at doses of 120 mg every 24 hours, ie, once daily (QD) to 700 mg BID were lower than observed on Day 1 after the first dose. The magnitude of the effect was greater at higher doses; lower doses of 15 mg BID and 60 mg BID did not show this effect. This observation may be related to the nonclinical finding that AG-348 is a potential inducer of human CYP3A4, an enzyme which biotransforms AG-348. It is possible that multiple doses of AG-348 leads to an increased rate of its own metabolism.

Evaluation of the effect of food on the PK of a single 700 mg dose of AG-348 in 5 subjects who were administered the drug while fasting and then, after an appropriate washout period, were re-administered the drug following ingestion of a standard United States Food and Drug Administration high-fat meal, showed that food likely has a minimal effect on the PK of AG-348.

#### 5.2.2.2. Pharmacodynamics

After a single dose of AG-348, a decrease in the concentration of 2,3-DPG was observed at 3 hours postdose, decreased in a dose-dependent manner to a minimum at 24-hour postdose, and then returned to values similar to baseline by 72 to 120 hours postdose. The mean decrease at 24 hours was approximately 300 µg/mL at the 700 through 2500 mg dose levels. Similar decreases were observed after the first dose of multiple doses and prior to doses in the MAD

study. After the final dose of multiple doses, the concentration of 2,3-DPG returned to values similar to baseline between 72 and 120 hours post dose.

After a single dose of AG-348, a minimal increase in the concentration of ATP was observed at 24 to 120 hours postdose.

In the MAD study, no increase was observed at 12 hours after the first dose; the concentration of ATP was increased on Day 8 to concentrations greater than in the SAD study, continued to trend upward on Day 11, and remained at a similar level through Day 14. The concentration of ATP remained elevated through 120 hours after the last dose on Day 14. The magnitude of the increase in ATP was similar across the dose range from 60 mg BID to 700 mg BID.

#### 5.2.2.3. Safety

Overall, AG-348 has been well-tolerated among healthy adult subjects at doses that produced strong pharmacodynamic (PD) effects on 2,3-DPG and ATP.

***Phase 1 Studies AG348-C-001 and AG348-C-002 in Healthy Adult Subjects***

After a single AG-348 dose, adverse events occurring in the treatment-emergent period (TEAEs) reported by > 1 subject at any time on study (either under fasted or fed conditions) included headache (22%), nausea (14%), and contact dermatitis and vomiting (each 6%). After repeated dosing of AG-348 for 14 days, TEAEs that occurred in > 5% of all AG-348-treated subjects across all cohorts included headache (13.9%), nausea (13.9%), vomiting (8.3%), decreased appetite (8.3%), feeling hot (8.3%), restlessness (8.3%), and dizziness, fatigue, vessel puncture site bruise, hyperhidrosis, dermatitis allergic, and drug eruption (5.6% each).

A dose-relationship was apparent with regard to the incidence of gastrointestinal events, primarily nausea and vomiting, with the incidence of such events increasing with increasing dose. Nausea and/or vomiting were observed only at doses ≥ 1400 mg in the SAD study and only at doses ≥ 700 BID in the MAD study. Nausea and vomiting were not observed at any dose ≤ 360 mg in either the SAD or MAD studies.

All but 1 TEAE reported to date have been mild or moderate (Grade 1 or 2) in intensity. The only Grade 3 TEAE was elevated liver transaminases (alanine aminotransferase [ALT]; aspartate aminotransferase [AST]) in a single subject in the MAD study treated with 700 mg AG-348 BID. The event was considered to be possibly related to AG-348, was declared a dose-limiting toxicity (DLT), and led to study drug discontinuation, after which the elevated liver transaminases resolved. Altogether, 3 of 6 subjects treated with AG-348 at 700 mg BID discontinued study drug in the MAD study: the subject described above with Grade 3 elevated transaminases, and 2 others who withdrew themselves for nausea and vomiting. A fourth subject treated with AG-348 at 60 mg BID was discontinued from the study for a drug eruption. No AG-348-treated subject discontinued in the SAD study due to an adverse event (AE).

Furthermore, no DLTs were observed after a single administration of AG-348 at doses of 30 mg to 2500 mg or after repeat administration of AG-348 for 14 days at daily doses of 15 mg to 700 mg BID except for the event of Grade 3 elevated liver function tests described above.

Due to preclinical observations pertaining to the potential for inhibition of the aromatase enzyme (see Section 5.2.1.4), the AG348-C-002 (MAD) study included assessment of baseline and serial measures of free and total serum testosterone and serum estradiol and estrone. In males treated

with AG-348, compared with placebo-treated males, the aromatase-dependent hormone assessments demonstrated an increase in total and free testosterone mean serum concentrations and decreased concentrations of estradiol and estrone at all doses of AG-348, including the lowest dose of 15 mg BID.

Most of the increases in total and free testosterone remained within the reference range, except at the 360 mg BID dose where the dose group means on Day 8 and Day 14 exceeded the upper limit of normal (ULN). Most of the estradiol concentrations observed in males remained within the reference range, but the mean male estrone concentrations dropped to the lower limit of quantification in all dose groups except 15 mg BID.

These changes in aromatase-dependent hormone levels in the male subjects treated with AG-348 are consistent with inhibition of human aromatase, and were reversible within 14 days upon cessation of dosing. The study did not enroll enough female subjects to draw any definitive conclusions regarding serial changes in aromatase-dependent hormones over time.

***Phase 2 Study in Adult Subjects with PK Deficiency***

As of a data cutoff of 27 March 2017, among the 52 subjects with PK deficiency who had received AG-348 in this study, 50 (96.2%) subjects had experienced at least 1 AE. Adverse events that occurred in ≥ 10% of subjects across both cohorts included headache (23 [44.2%] subjects); insomnia (20 [38.5%] subjects); nausea (19 [36.5%] subjects); viral upper respiratory tract infection (9 [17.3%] subjects); arthralgia and fatigue (8 [15.4%] subjects each); back pain, cough, dizziness, hot flush, and vomiting (7 [13.5%] subjects each); and diarrhea and influenza (6 [11.5%] subjects each).

Thirty-seven of 52 (71.2%) subjects who had received AG-348 experienced at least 1 AE that was considered by the Investigator to be study drug-related. Related AEs that occurred in ≥ 10% of AG-348-treated subjects included insomnia (17 [32.7%] subjects), nausea (15 [28.8%] subjects), headache (13 [25.0%] subjects), and hot flush (6 [11.5%] subjects).

The majority of AEs were assessed by the Investigator as National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Grade 1 or 2 in severity. There were 12 (23.1%) subjects with at least 1 reported AE that was CTCAE Grade ≥ 3. Adverse events that were Grade ≥ 3 included hypertriglyceridemia (3 [5.8%] subjects); anemia and insomnia (2 [3.8%] subjects each); and hemolysis, colitis, mesenteric vein thrombosis, influenza, pharyngitis, postprocedural hemorrhage, dizziness, and hypertension (1 [1.9%] subject each). Of these, 6 (11.5%) subjects had at least 1 Grade ≥ 3 AE that was considered study drug-related, including anemia and hypertriglyceridemia (2 [3.8%] subjects each) as well as hemolysis, pharyngitis, dizziness, and insomnia (1 [1.9%] subject each).

No deaths have been reported in this study.

A total of 9 (17.3%) subjects had experienced at least 1 SAE as of the data cutoff date. A total of 12 SAEs were reported in 9 subjects, including anemia in 2 (3.8%) subjects; and hemolysis, colitis, enteritis, mesenteric vein thrombosis, influenza, pharyngitis, cholelithiasis, postprocedural hemorrhage, osteoporosis, and musculoskeletal weakness in 1 (1.9%) subject each. Six SAEs were reported as being possibly or probably related to AG-348: osteoporosis, hemolysis, anemia (2 events), pharyngitis, and musculoskeletal weakness (the latter of which was determined to be nonserious after the data cutoff date). In addition, an SAE of

hypertriglyceridemia was reported as probably related to AG-348 but was not captured as treatment-emergent in the clinical database as of the cutoff date.

### 5.2.3. Summary of Overall Safety Management Plan

Measures to minimize the risks to subjects enrolled in the Core and Extension Periods have been taken with respect to the following study design elements:

- The initial doses for Arms 1 and 2 have been selected on the basis of safety and tolerability observations already carefully made in 2 predecessor clinical trials in adult healthy male and female subjects.
- The specified inclusion/exclusion criteria have been carefully considered to avoid enrollment of subjects for whom exposure to the study drug might pose a hazard.
- A designated Data Review Team (DRT) consisting of the overall study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer has been established to meet regularly at approximately 6-week intervals throughout the duration of the Core Period, and approximately every 4-6 months during the Extension Period once all subjects have completed the Core Period, to review the accumulating study data. The DRT will exercise options to suspend enrollment to 1 or both of the initial 2 study dose arms, discontinue enrollment to 1 or both of the initial 2 study dose arms, adjust the dose of subjects in 1 or both of the initial 2 study arms, and/or implement 1 new study dose arm.

  If a new dosing arm is implemented in the Core Period, the dose selected will not exceed 360 mg BID, the highest dose that demonstrated acceptable safety and tolerance in the 14-day, multiple-dose (BID) study in healthy adult subjects. Group cohort stopping rules for terminating enrollment into an arm based on the severity (CTCAE v4.03 grade) and frequency of AEs are defined.
- Dose modification and stopping rules are defined for individual subjects.
- Guidance for permitted, prohibited, and cautionary concomitant medications is specified based on the estimated potential for DDIs from hepatic cytochrome enzyme interactions with AG-348.
- Due to the potential for AG-348-mediated aromatase inhibition, bone mineral density will be monitored using dual-energy x-ray absorptiometry (DXA) scans (hip and spine) at Baseline (if subject has not had prior DXA scan within 3 months of Day 1) and between Week 24 and Week 28.

In the event that any clear and unequivocal, previously unidentified/unexpected toxicities occur in preclinical toxicology studies, the Sponsor will notify the Investigators, Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs), and regulatory authorities. The Sponsor will also report these to the DRT for review and discussion of appropriate actions, which may include, but are not necessarily limited to 1 or more of the following:

- Amending the protocol to adjust the inclusion/exclusion criteria (eg, to exclude subjects with certain at-risk concurrent conditions).

- Amending the protocol to adjust safety monitoring procedures (eg, to require additional monitoring of specified AEs, physical examinations, clinical laboratory testing, electrocardiogram [ECG] monitoring, or other testing as appropriate).
- Adjusting the dose of an arm of the study as appropriate.
- Adjusting the dose modification and/or stopping rules (Section 9.7).
- Adjusting the subject withdrawal criteria (Section 8.6).
- Terminating the trial.

### 5.2.4. Risks Associated with AG-348

#### 5.2.4.1. Risk of Withdrawal Hemolysis

Acute hemolysis upon sudden withdrawal of AG-348 has been observed in subjects with a marked and rapid increase in Hb. Investigators are advised to not discontinue dosing of AG-348 without first speaking with the study medical monitor for guidance on appropriate dose adjustment to avoid withdrawal hemolysis in subjects who experience a sustained increase in Hb. Any dose modification should be per protocol (Table 1).

#### 5.2.4.2. Risk of Insomnia

As discussed in Section 5.2.1.1 of this protocol and in the AG-348 Investigator's Brochure, AG-348 has been identified as a histamine H3 receptor antagonist/inverse agonist which has been documented to affect wakefulness and cognition in humans. No effects of histamine H3 modulation have been observed in safety pharmacology or toxicology studies (Schwartz 2011). Insomnia has been classified as an identified risk of AG-348 treatment, as described in the Investigator's Brochure.

#### 5.2.4.3. Risk of Bone Mineral Density Decrease

AG-348 inhibits human aromatase activity, as shown in studies in human placental microsomes and rat ovarian microsomes. In this study, 1 subject from 52 treated subjects experienced a medically important event of osteoporosis (Grade 2), which was assessed as likely due to aromatase inhibition.

#### 5.2.4.4. Potential Risk of Transaminase Increases

Transient increases in transaminases have been observed with AG-348, as described in the Investigator's Brochure. No SAEs or cases of suspected drug-induced liver injuries have been reported. Liver function test results should be monitored per protocol. Transaminase increase is an AE of special interest (AESI) for AG-348 (refer to Section 11.1.5 for details).

#### 5.2.4.5. Potential Risk of Triglyceride Increases

Transient increases in triglycerides have been observed with AG-348, as described in the Investigator's Brochure. One observed case was an SAE that recovered after AG-348 discontinuation. No cases of pancreatitis have been reported.

#### 5.2.4.6. Potential Risk of Photosensitivity

AG-348 may cause sensitivity to direct and indirect sunlight. Subjects should be warned to avoid direct sun exposure. When exposure to sunlight is anticipated for longer than 15 minutes, the subject should be instructed to apply Sun Protection Factor 30 or higher sunscreen to exposed areas and wear protective clothing and sunglasses.

### 5.2.5. Other Restrictions and Precautions

Subjects should be advised to refrain from altering their normal exercise routine for the first 28 days of treatment with AG-348, as symptoms of anemia could theoretically temporarily worsen before the eventual potential beneficial effect of AG-348 on the Hb level becomes evident.

The elevated blood levels of 2,3-DPG commonly seen in subjects with PK deficiency may produce a right shift in the Hb-O2 dissociation curve. The effect of this is to enhance oxygen delivery at the tissue level, thus counteracting to some extent the physiologic consequences of the anemia. AG-348 was shown to produce rapid decreases (within the first 12 hours following a single dose) in 2,3-DPG in 2 prior clinical trials with healthy adult male and female subjects. In subjects with PK deficiency who have elevated 2,3-DPG levels, it is theoretically possible that decreases in 2,3-DPG that precede correction of the anemia could result in a temporary decrease of oxygen delivery at the tissue level resulting in clinical symptoms (eg, increased fatigue).

Subjects should not discontinue dosing without first speaking with the treating Investigator; abrupt discontinuation of AG-348 dosing in a subject who experience a substantial increase in Hb may result in withdrawal hemolysis.

# 6. STUDY OBJECTIVES AND ENDPOINTS

## 6.1. Core Period

### 6.1.1. Primary Objective

The primary objective of the Core Period of the study is to:

- Evaluate the safety and tolerability of up to 24 weeks of AG-348 administration in subjects with PK deficiency.

### 6.1.2. Secondary Objectives

The secondary objectives of the Core Period of the study are to:

- Evaluate the PK of AG-348 and the metabolite AGI-8702.
- Evaluate the PD response of ATP and 2,3-DPG after administration of AG-348.
- Evaluate indicators of clinical activity of AG-348 in subjects with PK deficiency, including changes in Hb, HCT, reticulocyte count, haptoglobin (Hp), carboxyhemoglobin (COHb), lactate dehydrogenase (LDH), total and indirect bilirubin, erythropoietin (EPO), hepcidin, ferritin, and transferrin saturation (serum iron/iron-binding capacity).

## 6.2. Extension Period

### 6.2.1. Primary Objective

The primary objective of the Extension Period of the study is to:

- Evaluate the safety and tolerability of up to 30 months of AG-348 administration in subjects with PK deficiency.

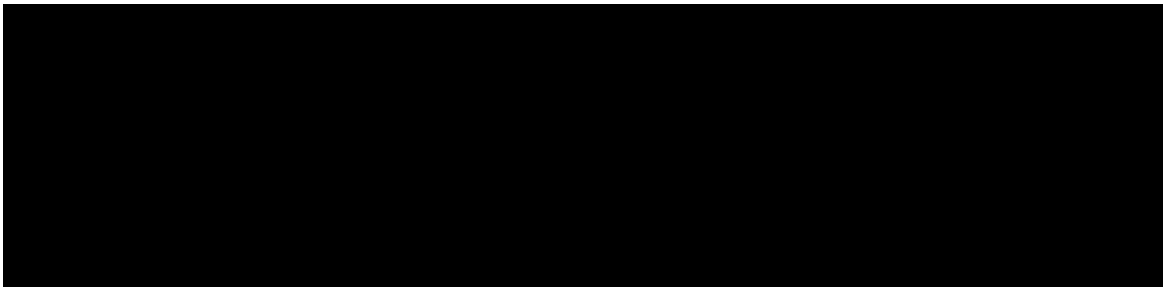
### 6.2.2. Secondary Objectives

The secondary objectives of the Extension Period are to:

- Evaluate the PK of AG-348 and the metabolite AGI-8702.
- Evaluate the PD response of ATP and 2,3-DPG after administration of AG-348.
- Evaluate indicators of clinical activity of AG-348 in subjects with PK deficiency, including changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and

indirect bilirubin, EPO, hepcidin, ferritin, and transferrin saturation (serum iron/iron-binding capacity).

- Evaluate the optimal maintenance dose of AG-348 for each individual subject during the Extension Period.



## 6.3. Study Measures and Endpoints

### 6.3.1. Safety Measures and Endpoints

Safety will be evaluated by all of the following:

- Monitoring of AEs, including SAEs, AESIs, and AEs leading to discontinuation. All AEs will be graded using CTCAE, Version 4.03.
- Safety laboratory parameters (hematology, chemistry, urinalysis, coagulation).
- Physical examination findings (including neurological examination).
- Vital signs (VS).
- 12-lead electrocardiograms (ECGs).
- DXA scans.
- Serum sex hormone levels (testosterone [total and free], estrone, and estradiol), bone turnover markers (serum osteocalcin-N-mid and serum C-terminal telopeptide [CTX]), 25-hydroxy vitamin D2 and D3, total cholesterol, high-density lipoprotein-cholesterol (HDL-C), and triglycerides will be monitored for evidence of potential inhibition of aromatase by AG-348.
- Menstruating female subjects will also keep a paper-based menstrual cycle diary throughout the Core and Extension Periods.

### 6.3.2. Clinical Activity Measures and Endpoints

- Monitoring of potential indicators of clinical activity will include evaluating changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, hepcidin, ferritin, and transferrin saturation.

### 6.3.3. Pharmacokinetic and Pharmacodynamic Measures and Endpoints

The PK and PD profile of AG-348 will be evaluated by all of the following:

- Approximately the first 10 subjects treated during the Core Period, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 1.3. The remainder of treated subjects will undergo limited PK sampling as detailed in Appendix 1.4.
- During the Core Period, serial blood sampling for determination of concentration-time profiles of AG-348 and its metabolite AGI-8702 will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of AG-348 and AGI-8702 will be obtained.
- During the Extension Period, predose PK samples will be drawn for the measurement of trough levels of AG-348 and AGI-8702 at each study visit (every 3 months; Appendix 1.2). AG-348 and AGI-8702 will be analyzed using qualified assays to determine concentrations in plasma.
- Pharmacodynamic assessments during the Core Period will include 2,3-DPG, ATP (secondary objectives), [redacted]

  Approximately the first 10 subjects treated during the Core Period will undergo extensive PD sampling as detailed in Appendix 1.3. The remainder of treated subjects will undergo limited PD sampling as detailed in Appendix 1.4.
- During the Core Period, serial blood sampling for determination of levels of ATP and, 2,3-DPG will be conducted following the first dose and the morning Day15 dose, and additional trough levels of ATP and 2,3-DPG will be obtained.
- During the Extension Period, predose PD samples will be drawn for the measurement of trough levels of 2,3-DPG, ATP, [redacted] at each study visit (every 3 months; Appendix 1.2). Adenosine triphosphate and 2,3-DPG will be analyzed using qualified assays to determine concentrations in whole blood.

[redacted]

# 7. INVESTIGATIONAL PLAN

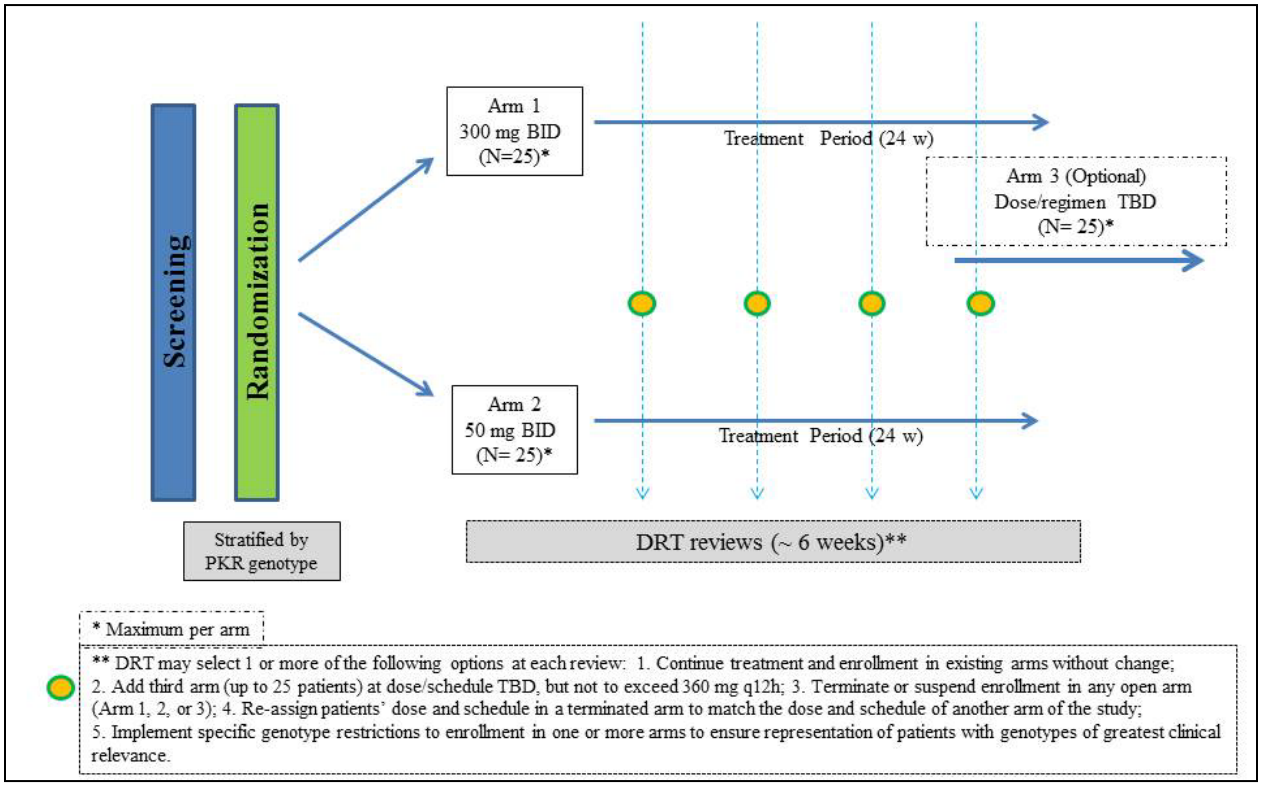
## 7.1. Overall Study Design

Study AG348-C-003 is a Phase 2, open-label, 2-arm, multicenter, randomized, dose-ranging study in adult subjects with PK deficiency; the study will be divided in to a Core Period and an Extension Period. During the Core Period, subjects will receive multiple doses of AG-348 for up to 24 weeks; subjects who are eligible can enter the Extension Period to receive AG-348 for up to 2 years following the end of the Core Period.

Subjects with PK deficiency confirmed by RBC PK enzymatic assay performed at Screening will be eligible to participate in this study. At the Week 24 visit, subjects who safely tolerate AG-348 and demonstrate clinical activity of AG-348 may be eligible to immediately roll over to the Extension Period for continued treatment. Subjects who complete treatment at the end of the Core Period (24 weeks) will undergo follow-up assessment 4 weeks after the last dose of study drug. If a subject discontinues at any other time (including discontinuation during the Core or Extension Period), follow-up assessments will be conducted 4 weeks after discontinuation. Subjects with toxicity suspected to be related to study drug will continue follow-up until the AE resolves, is declared chronic by the Investigator, or the subject is lost to follow-up.

For the Core Period, up to 25 subjects will be initially randomized on an open-label, 1:1 basis to each of 2 BID doses of AG-348 (up to 50 subjects; Figure 2).

**Figure 2: Study Schema: Core Period**




Abbreviations: BID (q12h) = twice-daily (every 12 hours); DRT = data review team; PKR = pyruvate kinase red blood cell isoform; TBD = to be determined; w = weeks.

The dose of Arm 1 is 300 mg of AG-348 administered PO q12h (ie, BID). The dose of Arm 2 is 50 mg of AG-348 administered PO BID. Randomization will be stratified by *PKR* mutation in order to maintain balance as much as possible across the dose arms for the specific mutations expected to be most frequently enrolled. The PKR mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H, and all other mutations as "other"). Mutation status is defined by the presence of at least 1 of the indicated mutations; subjects with more than 1 stratified mutation will be assigned based on Sponsor's discretion.

The doses for each arm of the Core Period have been selected from the AG348-C-001 SAD study and AG348-C-002 MAD studies in healthy adult subjects to represent the range of doses/exposures that were safely tolerated and resulted in maximal or near maximal PD effects on 2,3-DPG and ATP.

Because PK deficiency is a rare disease with a limited eligible subject population and because the underlying pathophysiology and clinical phenotype of affected subjects is heterogeneous due to the wide variety of mutations in PKR that cause the disease, it is deemed important to focus closely on dose findings in this first-inpatient study. Therefore, in addition to initiating this study with 2 different doses of AG-348 administered BID, a DRT will be established to review study data on a frequent basis and adapt the study design, dose and schedule of AG-348 as indicated.

The DRT will monitor safety on an ongoing basis and meet at regular intervals of approximately every 6 weeks, or ad hoc as necessary, for as long as any subjects are still in the Core Period to review AEs, VS, clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and ECGs. The DRT will also review available PK/PD data and indicators of clinical activity (eg, changes from baseline in Hb). These DRT meetings will also include data review for all subjects that may be under treatment in the Extension Period.

If there are no subjects still being treated in the Core Period, and the only subjects on treatment are those in the Extension Period, then the frequency of the DRT meetings will reduce to approximately every 4 to 6 months in order to match the frequency of subject visits (and new data collection) in the Extension Period. When all the subjects are in the Extension Period, PK/PD data will no longer be reviewed by the DRT. The DRT will be comprised of the study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer.

Beginning 6 weeks after the first subject is dosed in the Core Period or ad hoc as necessary, and proceeding according the schedule indicated above (approximately every 6 weeks during the Core Period, approximately every 4 to 6 months during the Extension Period once all subjects have completed the Core Period), the DRT will review cumulative safety data, available PK/PD data, and clinical activity data.

Based on the DRT's recurring, the DRT may exercise 1 or more of the following options during the Core Period:

- Continue treatment and enrollment in existing arms without change.
- Add 1 new dose arm (Arm 3) to enroll up to 25 subjects at a dose to be determined; the dose for Arm 3 may be lower or higher than Arm 1 and Arm 2 doses, but will not exceed 360 mg BID; and the dose regimen may be less frequent than BID.

- Terminate or suspend enrollment to allow further review of clinical data in Arm 1 and/or Arm 2 (and/or potential Arm 3).

  Enrollment in an arm could be terminated or suspended to allow further review, for example, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity.

- Re-assign subject's doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the subjects in the terminated arm will remain in their original arm, ie, they will not count towards the enrollment quota of the arm whose dose and schedule is being adopted.

- Implement specific genotype restrictions to enrollment in 1 or more arms to ensure representation of subjects with genotypes of greatest clinical relevance.

The DRT may exercise 1 or more of the following options during the Extension Period:

- Continue treatment without change.

- Re-assign subjects' doses and schedule to an existing dose and schedule that has been determined to be safer, and/or have a better PD response, or produce signs of clinical activity.

- Terminate or suspend treatment to allow further review of clinical data (eg, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity).

The data that the DRT will review to make these decisions are expected to include, but are not necessarily limited to, the following:

- *Safety Observations:* All AEs, VS, clinical laboratory assessments (hematology, clinical chemistry, coagulation, and urinalysis), and ECGs.

- *PK and PD Observations:* Includes changes in 2,3-DPG and ATP, except when all subjects are in the Extension Period.

- *Indicators of Clinical Activity:* Includes changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, EPO, hepcidin, total and indirect bilirubin, ferritin, and transferrin saturation.

If a third dose arm is implemented, the dose of AG-348 selected will not exceed 360 mg BID, as this was the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy adult subjects. The PK/PD sampling schedule in a potential third dosing arm will be determined by Sponsor's discretion and may follow either the extensive or limited PK/PD sampling schedules as specified.

Subjects in the Extension Period will undergo a gradual dose-taper regimen to identify the optimal maintenance dose for each (defined as the dose that results in ≤ 1.0 g/dL decrease in Hb compared to the pre-taper Hb value on at least 2 measurements following each dose taper step) on a per-subject basis (Section 9.7.3).

Due to the potential for AG-348-mediated aromatase inhibition, combined with the known risk of osteoporosis in subjects with congenital hemolytic anemias (Steer, et al. 2017; Wong, et al. 2014), DXA scan (hip and spine) will be performed at Screening (if subject has not had prior DXA scan within 3 months of Day 1) to obtain bone mineral density and T and Z scores. These data are intended to serve as a baseline measure of bone mineral density for all enrolling subjects, and are deemed of particular importance for those who may enter the longer-term Extension Period after completing 24 weeks of treatment (Core Period). All subjects will have a second DXA scan in the interval between Weeks 24 and 28 for the Core Period. Subjects in the Extension Period will have additional DXA scans at Months 12, 18, 24, and 30.

As the number of enrolling arms changes in the study (eg, from 2 to 3), the randomization scheme will adjust to enable balanced randomization into each actively accruing arm. Randomization and stratification will cease in the event that only a single arm is left enrolling.

Depending on possible early termination of 1 or both of the initial 2 arms, or the addition of a third arm, the study could enroll up to a maximum of 75 subjects.

The Investigator will monitor all subjects for safety and tolerability. Modification of an individual subject's dose of AG-348 will be based on AEs and observed changes in Hb levels as detailed in Section 9.7.1 and Section 9.7.2.

Screening assessments will occur within 42 days prior to the first dose of study treatment. During the Core Period, subjects will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12), and monthly through Week 24 (Weeks 16, 20, and 24). Subjects who safely tolerate AG 348 through Week 24 (Core Period) may be eligible to immediately enter the Extension Period for continued treatment.

Study visits for safety and clinical activity assessments will occur approximately every 3 months during the Extension Period for up to 2 years after the end of the Core Period, except during the dose taper part of the Extension Period when weekly visits will be performed (see Section 9.7.3.1 for details). All subjects will undergo a follow-up assessment 4 weeks after the last dose of AG-348, regardless of whether this was due to discontinuation, the last dose in the Core Period for a subject who chooses not to continue in the Extension Period, or the last dose of the Extension Period except for those who may continue treatment in an extension study (to be designed) after having completed the Extension Period.

Safety assessments will include monitoring of AEs, including SAEs, AESIs, and AEs leading to discontinuation; safety laboratory parameters (eg, hematology, serum chemistry, coagulation studies, and urinalysis); physical examination findings (including neurological examination); VS; 12-lead ECGs, and DXA scans. Additional safety assessments will include monitoring of sex hormone levels (testosterone [total and free], estrone, and estradiol), bone turnover markers (osteocalcin-N-mid and CTX), 25-hydroxy vitamin D2 and D3 levels, total cholesterol, HDL-C, and triglycerides.

Follow-up assessments will be conducted on Day 197 (Week 28) for subjects who do not enter the Extension Period and will include physical examination, weight, performance status, VS, 12-lead ECGs, laboratory evaluations (hematology, Hp, EPO levels, serum chemistry, coagulation studies, urinalysis; lipids, hormonal testing), AEs, and transfusion record.

These follow-up assessments will be performed approximately 4 weeks after discontinuation of AG-348 for subjects who discontinue prior to completion of the Core Period and for those who discontinue in the Extension Period. Menstruating female subjects will also be required to keep a paper-based menstrual cycle diary throughout the study.

Pharmacokinetic assessments will include serial blood sampling for PK profiles of AG-348 and its metabolite AGI-8702.

Pharmacodynamic evaluations will include serial blood sampling for determination of levels of ATP and 2,3 DPG. Extensive PK/PD sampling will be conducted on the first approximately 10 subjects total treated in Arms 1 and 2 of the Core Period (Appendix 1.3) while limited PK/PD sampling will be conducted on the remainder of treated subjects (Appendix 1.4). Limited trough sampling will be conducted every 3 months during the Extension Period (Appendix 1.2).

## 7.2. Rationale for the Study Design

The primary and secondary objectives of this study are to evaluate the safety, tolerability, PK and PD, and indicators of clinical activity of AG-348 in subjects with PK deficiency. The choice of dose and schedule of administration of AG -348 for Arms 1 and 2 was based on the highest safely tolerated dose (Arm 1: 300 mg BID) and the lowest dose with potentially relevant PD activity (Arm 2: 50 mg BID) from the forerunner AG348-C-002 MAD study in healthy adult subjects.

Decisions regarding continuing enrollment and treatment in these initial dose arms and/or implementation of an additional dose arm will be based on DRT review of safety, PK and PD data, and indicators of clinical activity collected from all subjects treated in Arm 1 and Arm 2. This design was chosen to minimize risk to subjects while allowing evaluation of safe and pharmacologically active dose levels of AG-348, and to allow the necessary flexibility to adjust dose and schedule should the safety, tolerability, PK, and/or PD be different in subjects with PK deficiency compared with healthy adult subjects.

Additional safety measures intended to minimize risk to subjects include monitoring of AEs by the DRT and specified provisions for individual subject dose modification as needed for safety and (potentially) large increases in Hb level (Section 9.7.1 and Section 9.7.2). Measures intended to maximize the opportunity for subjects with demonstrated safety and tolerability to continue to derive benefit from any observed clinical activity of AG-348 include the option for continued treatment in the Extension Period.

A comprehensive series of safety evaluations, including laboratory parameters, physical examinations (including neurological examination), VS, 12-lead ECGs, and monitoring for SAEs and AEs, will be conducted to evaluate the safety profile of AG-348 and to aid in the determination of the recommended dose for continued development.

The study includes serial blood sampling across at least 2 different doses of the study drug to assess its PK and PD profiles.

Consistent with the design of many Phase 2 studies, preliminary evaluation of the potential PD and clinical activity of AG-348 are secondary objectives of this study. The latter will include assessments of the PK/PD relationship between AG-348 and the biomarkers ATP and 2,3-DPG, [redacted]

## 7.3. Rationales Related to Dosing

### 7.3.1. Rationale for the Starting Dose

Prior to execution of this study, the Sponsor conducted 2 clinical studies of AG-348 in healthy adult subjects, including a SAD study (AG348-C-001) and a MAD (14-day, BID) study (AG348-C-002). Available details of these studies are discussed in the current Investigator's Brochure (IB). Between these 2 studies, 72 healthy human subjects have been dosed with AG-348. In vitro investigations, also reported in the Investigator's Brochure, had previously demonstrated that AG-348 increased the activity of WT PKR approximately to the same extent as it did a series a recombinant mPKRs. Therefore it was deemed reasonable to study the safety, tolerability, PK, and PD of AG-348 in healthy subjects in a controlled Phase 1 setting as a more efficient means of obtaining information than in the actual rare disease population of subjects with PK deficiency.

The MAD study demonstrated that the exposures produced by AG-348 doses from 60 mg BID to 360 mg BID (including 120 mg QD) resulted in maximal changes from baseline for the PD markers 2,3-DPG (reduction from baseline) and ATP (increase from baseline). The exposures resulting from doses less than 60 mg BID were of lesser magnitude and the exposures resulting from doses greater than 360 mg BID were of no greater magnitude than the aforementioned range. Therefore the starting doses for this first dose ranging study in subjects with PK deficiency were selected to be 300 mg BID (Arm 1) and 50 mg BID (Arm 2). These doses were demonstrated to be safe and tolerable in the healthy adult subject studies.

### 7.3.2. Rationale for the Dose Range

The availability of ATP is proposed as being critical for optimally maintaining RBC membrane integrity (Section 5.1). The dose ranges from 50 mg BID to 300 mg BID may result in clinically effective modulation of PKR in PK deficiency subjects if the mutated enzyme is responsive to AG-348 in a similar manner to the WT enzyme in healthy subjects. However, there are many different mutations in PKR that result in PK deficiency, and these mutations produce variable effects on the enzyme in terms of catalytic activity and thermal stability. It is not known if different mutations will respond clinically in a similar manner to the same exposure to AG-348. Therefore, it is prudent to study the range of safe and pharmacodynamically relevant doses as specified in this study, and to allow flexibility for the DRT to analyze the evolving study data to adapt the dose and schedule of administration of AG-348 to produce the optimal combination of safety, tolerability, and PD, and, potentially, clinical response.

### 7.3.3. Rationale for the Duration of Dosing

The initial treatment duration of 24 weeks (6 months) for the Core Period was chosen for this proof-of-concept trial for 2 principal reasons: 1) to begin establishing a safety database addressing the chronic administration of AG-348; and 2) to allow sufficient time for clinical response to treatment to appear.

It is anticipated that this treatment, if successful, may be taken for life, as PK deficiency is a genetically determined inborn error of metabolism. Therefore, it is important to begin to investigate the long-term safety of the treatment. As will be discussed below, the safety package supports the treatment duration of 6 months.

Red blood cell turnover is typically 120 days (4 months), although it may be shorter in some populations of pyruvate kinase deficient RBCs (Mentzer, et al. 1971). Different PKR mutations produce a variety of physiologic consequences. In mutations where normal or nearly normal levels of PKR protein persist, but the protein catalytic function is impaired, an improvement in PKR functional activity might be seen relatively quickly upon exposure to AG-348. However, in cases where the PKR mutation results in an unstable mutant, PKR protein levels may be low and additional time may be required for stabilization of the newly synthesized mutant enzyme in developing bone marrow erythroblasts so that eventually more mature RBCs may be produced with more nearly normal levels of functional PKR protein.

This study plans up to 24 weeks of dosing with AG-348 during the Core Period with the possibility of continued dosing beyond 24 weeks during the Extension Period, up to 2 years, in subjects for whom AG-348 is safely tolerated and demonstrates clinical activity.

The International Conference on Harmonisation (ICH) Guideline M3(R2) on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals recommends that clinical trials of a duration between 2 weeks and 6 months should be supported by repeat-dose toxicity studies of at least equivalent duration, and clinical trials lasting beyond 6 months should be supported by 6-month rodent and 9-month nonrodent studies. This guidance allows for clinical trials of longer duration to be initiated based on 3 months of nonclinical data, and for clinical dosing in serious or life-threatening indications to be extended based on complete chronic rodent data and in-life and necropsy data in the nonrodent (and complete histopathology in the nonrodent provided within an additional 3 months).

For the current investigational product (AG-348), 13-week, repeat-dose toxicology studies have been completed and are summarized in Section 5.2.1.4 of this protocol and in the current Investigator's Brochure. Considering that PK deficiency is a serious condition with an unmet medical need, it is appropriate to initiate this clinical study with the available nonclinical data.

### 7.3.4. Rationale for Identification of Individual Optimal Maintenance Dose and Gradual Dose-Taper Regimen

AG-348 has proven efficacious across a range of doses in this study, with several subjects having achieved and maintained clinically meaningful increases in Hb at doses < 50 mg BID. Previously, dose decreases have been implemented in this study only for safety/tolerability reasons and in subjects with Hb values reaching > 13.5 g/dL (for women) or > 15.0 g/dL (for men).

In subjects who have a sustained increase in Hb while continuing to tolerate their randomized dose of either 50 mg BID or 300 mg BID, there is no provision in the current protocol for dose de-escalation. However, it is possible and even likely that some subjects may maintain improved Hb values at a lower dose of AG-348.

A general principle in medicine is to administer the lowest efficacious dose of a drug. Furthermore, it is expected that administration of lower doses of AG-348 will be associated with reduced frequency and severity of AEs.

Based upon these considerations, the protocol will now include the practice of attempting to decrease each subject's dose while maintaining a desirable Hb level.

Subjects remaining on study are receiving AG-348 across a wide range of doses (from 300 mg BID to 5 mg QD). These observations indicate that the optimal maintenance dose of AG-348 is likely to vary by subject and cannot be determined in advance. Consequently, individual optimal maintenance doses will be determined empirically by an individualized gradual dose taper while monitoring Hb and Hp levels.

An additional justification for implementing a gradual dose taper is the finding that abrupt cessation of treatment with AG-348, which was implemented for 2 responsive subjects in the Core Period with Hb values over the protocol-defined threshold, resulted in withdrawal hemolysis and anemia. Therefore, it is not known whether a sharp decrease in AG-348 dosing (eg, from 300 mg BID to 50 mg BID) would have the same effect as a treatment interruption; however, the scenario is theoretically possible in subjects with an individual optimal maintenance dose > 50 mg BID.

## 7.4. Criteria for Study Termination

This study may be prematurely terminated if, in the opinion of the Sponsor, there is sufficient reasonable cause. In the event of such action, written notification documenting the reason for study termination will be provided to each Investigator.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects.
- Insufficient adherence to protocol requirements.
- Plans to modify, suspend, or discontinue the development of the study drug.
- Other administrative reasons.

Should the study be closed prematurely, all study materials must be returned to the Sponsor or the Sponsor's designee. Subjects who have participated in the Extension Period may be able to screen for eligibility in a study for a second generation allosteric PKR activator, if such a compound is available at that time, and after a suitable washout period has been observed.

# 8. STUDY POPULATION

## 8.1. Number of Subjects

Up to approximately 75 subjects may be enrolled in this study.

## 8.2. Inclusion Criteria

### 8.2.1. Core Period

Subjects must meet all of the following criteria to be eligible for inclusion in the Core Period of the study:

1. Have provided signed written informed consent prior to undergoing any study procedure, including Screening procedures.
2. Be male or female, aged 18 years and older.
3. Have a known medical history of PK deficiency.
4. Have documented clinical laboratory confirmation of PK deficiency by RBC pyruvate kinase enzymatic assay performed at Screening, either by a designated central laboratory or by any participating investigative site's local hematology laboratory. Subjects with prior documentation of PK deficiency by RBC enzymatic assay must have reconfirmation of this result during Screening as a condition of enrollment.

   NOTES:

   i. In the event that a subject's Screening pyruvate kinase enzymatic assay is negative (ie, shows normal pyruvate kinase activity), the subject will be eligible for enrollment if the genotyping shows a mutant genotype that has been previously documented in the literature to be associated with PK deficiency.

   ii. If the genotyping shows a previously undescribed mutation in the PKR gene, the subject's eligibility for enrollment will be determined on an individual case basis by the Coordinating Investigator and Medical Monitor in discussion with the Investigator.

   iii. If no mutation is defined, the subject will not be eligible for enrollment.
5. Have a blood sample for genotypic characterization of the mutant PKR gene performed by the designated central laboratory at Screening.

   NOTES:

   i. The designated central laboratory-determined genotype will generally serve as the basis for genotyping for enrollment. However, subjects whose genotype has already been determined by another laboratory may be enrolled on the basis of that report, with the approval of the Medical Monitor, in the case of an unexpected delay in return of the designated central laboratory result during the Screening Period.

ii. Enrollment on the basis of a result from a laboratory other than the designated central genotyping laboratory does not relieve the inclusion requirement that ALL subjects must have a sample sent to the designated central genotyping laboratory.

6. Have Hb ≤ 12.0 g/dL (if male) or ≤ 11.0 g/dL (if female).
7. Be considered transfusion-independent, defined as having had ≤ 3 units of RBCs transfused in the 12-month period up to the first day of study drug dosing and no transfusions within 4 months of the first day of study dosing.

   NOTE: Subjects who have received more transfusion support than described above will be evaluated for eligibility on a case-by-case basis by the Medical Monitor.
8. Have their spleen in place or have undergone splenectomy. Splenectomized subjects must meet all of the following conditions:
   i. Have undergone the procedure ≥ 6 months prior to Screening.
   ii. Be current in their vaccinations for pneumococcal conjugate (PCV13), pneumococcal polysaccharide (PPSV23), quadrivalent meningococcal vaccine, and *Haemophilus influenzae* Type B, as recommended by the United States Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices (refer to http://www.cdc.gov/vaccines/schedules/downloads/adult/adult-schedule.pdf) or, for subjects in Canada or the European Union, by immunization advisory groups in those locations.

      NOTE: Any missing vaccinations may be administered, starting with the Screening Period and continuing throughout the trial, following the initiation of AG-348 dosing and as necessary according to recommended vaccination guidance.
9. Have Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 2.
10. Have been taking ≥ 1 mg of folic acid daily for ≥ 21 days prior to the first dose of study drug and agree to continue this regimen during the study.
11. Have adequate organ function, defined as meeting all of the following conditions:
    i. Serum AST ≤ 2.5 × ULN, unless the increased AST is assessed by the Investigator as due to hemolysis and/or hepatic iron deposition, and ALT ≤ 2.5 × ULN, unless the increased ALT is assessed by the Investigator as due to hepatic iron deposition.
    ii. Either normal or elevated levels of serum bilirubin. In subjects with serum bilirubin > ULN, the elevation must be attributed to hemolysis with or without Gilbert's syndrome and must not be attributed to choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease.
    iii. Serum creatinine ≤ 1.25 × ULN or, if > 1.25 × ULN, then 24-hour measured or calculated (by Cockcroft-Gault) glomerular filtration rate ≥ 60 mL/min.
    iv. Absolute neutrophil count (ANC) ≥ $1.0 \times 10^9$/L.

v. Platelet count $\geq 100 \times 10^9$/L.

vi. Activated partial thromboplastin time (aPTT) and international normalized ratio (INR) $\leq 1.25 \times$ ULN, unless the subject is receiving therapeutic anticoagulants.

12. For women of childbearing potential—defined as females who either have experienced menarche and have not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, and/or bilateral oophorectomy) or who are not postmenopausal (defined as either having amenorrhea for ≥ 12 consecutive months without another cause and documented serum follicle-stimulating hormone [FSH] level > 35 mIU/mL or being ≥ 62 years of age and having amenorrhea for ≥ 12 consecutive months [no FSH testing required]):

    i. Agree to abstain from sexual intercourse or to use an acceptable/effective method of contraception (ie, condom plus spermicide, condom plus oral contraceptive, condom plus intrauterine device, condom plus diaphragm with spermicide) from as soon as is feasible during the Screening period until 30 days following the last dose of AG-348.

    NOTE: Abstinence is an acceptable method only when this is in line with the normal lifestyle of the subject, meaning that the subject plans to remain abstinent *continuously* throughout the duration of the study and for ≥ 30 days after the last dose of study drug. Periodic abstinence (eg, calendar, symptothermal, and postovulation methods) and withdrawal are not acceptable methods of contraception.

    ii. Have negative serum or urine pregnancy test within 72 hours before start of AG-348 dosing.

    iii. Not be breastfeeding.

13. If male (with the exception of subjects who have undergone vasectomy ≥ 6 months prior to Screening), agree to abstain from sexual intercourse or, if sexually active, to use a condom with spermicide as contraception (regardless of their female partner's childbearing potential or their partner's use of their own contraception) from Day 1 of dosing until 30 days following the last dose of AG-348.

    NOTE: Abstinence is an acceptable method only when this is in line with the normal life style of the subject, meaning that the subject plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence (eg, selective timing of intercourse based on partner’s calendar, symptothermal, and postovulation methods) and withdrawal are not acceptable methods of contraception.

### 8.2.2. Extension Period

Subjects must meet all of the following criteria to be eligible for inclusion in the Extension Period of the study:

1. Have provided signed written informed consent prior to undergoing any study procedure during the Extension Period.
2. Have completed 24 weeks of treatment during the Core Period and tolerated AG-348 (defined as having completed 24 weeks with or without protocol-permitted dose modifications).
3. The treating Investigator agrees that there is a potential for clinical benefit to the subject from continued treatment and recommends participation in the Extension Period.
4. The Sponsor's designated Medical Monitor or Responsible Medical Officer approves the subject's participation in the Extension Period.
5. If applicable, agree to continue to follow the same sexual abstinence/contraception rules as stated in Section 8.2.1, Inclusion Criterion 12 (for females) or 13 (for males).

## 8.3. Exclusion Criteria

### 8.3.1. Core Period

Subjects who meet any of the following criteria will be excluded from the Core Period of the study:

1. Have Hb level > 12.0 g/dL (if male) or > 11.0 g/dL (if female).
2. Have an additional diagnosis of any other congenital or acquired blood disorder, including glucose-6-phosphate-dehydrogenase (G6PD) deficiency, or any other hemolytic anemia process, with the exception of mild allo-immunization as a consequence of transfusion therapy.
3. Have iron overload (hemosiderosis or concurrent hemochromatosis) sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac, hepatic, or pancreatic insufficiency.
4. Have had prior bone marrow or stem cell transplant.
5. Have clinically symptomatic cholelithiasis or cholecystitis.

   NOTES:

   i. Prior cholecystectomy is not exclusionary.

   ii. Subjects with symptomatic cholelithiasis or cholecystitis may be re-screened once the disorder has been treated and clinical symptoms have resolved.

6. Be currently enrolled in another therapeutic clinical trial involving ongoing therapy with any investigational or marketed product or placebo.

   NOTE: Concurrent participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480) is permitted.

7. Have been exposed to any investigational drug, device, or procedure within 28 days prior to Screening or during trial participation.
8. Have any concurrent medical condition that could compromise participation in the study, such as:
    i. Poorly controlled hypertension (defined as systolic blood pressure [BP] > 150 mm Hg or diastolic BP > 90 mm Hg) refractory to medical management.
    ii. History of recent (within < 6 months from Screening date) congestive heart failure; myocardial infarction or unstable angina pectoris; hemorrhagic, embolic, or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.
    iii. Currently active infection requiring the use of parenteral antimicrobial agents or of ≥ Grade 3 severity (per CTCAE v4.03) within 6 months of first dose of study drug.
    iv. Pattern or frequency of postsplenectomy sepsis that, in the assessment of the Investigator, could reasonably be expected to interfere with the ability of the subject to complete participation in the 24-week Core Period of the study.
    v. Positive test for hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV) antibody with accompanying signs of active hepatitis B or C infection.
    vi. Positive test for human immunodeficiency virus (HIV) 1 or 2 antibody.
    vii. Diabetes mellitus that is judged to be in poor control by the Investigator or which requires > 3 anti-diabetic agents, counting insulin (all insulins are considered 1 agent).

        NOTE: Use of insulin per se is not exclusionary.
    viii. History of any primary malignancy, with the exception of: curatively treated nonmelanomatous skin cancer, curatively treated cervical or breast carcinoma in situ, or any other primary tumor treated with curative intent, and with no known active disease present and no anticancer treatment administered during the last 3 years.
9. Have undergone major surgery within 6 months of first dose of study drug.
10. Have currently or have a recent history of a psychiatric disorder that, in the opinion of the Investigator or Medical Monitor, could compromise the ability of the subject to cooperate with study visits and procedures.
11. Have used any of the restricted list of products known to strongly inhibit cytochrome P450 (CYP) 3A4 drug metabolism (Appendix 4.1) within 5 days prior to Day 1 dosing; products known to strongly induce CYP3A4 metabolism (Appendix 4.2) within 28 days prior to Day 1 dosing; products known to strongly inhibit P-glycoprotein (P-gp) transporter (Appendix 4.3) within 5 days prior to Day 1 dosing; or digoxin within 5 days prior to Day 1 dosing.

12. Have serum bilirubin > ULN attributable to factors other than hemolysis and/or Gilbert's syndrome.
13. Have heart-rate corrected QT interval by Fridericia's method (QTcF) > 450 msec (for males) or > 470 msec (for females), with the exception of subjects with a left bundle branch block (LBBB), for whom Medical Monitor approval is needed to enroll.
14. Have cardiac dysrhythmia that is judged clinically significant by the Investigator or which requires therapy with drugs that are primarily substrates of CYP3A4.
15. Have any history of allergy to sulfonamides characterized by acute hemolytic anemia, anaphylaxis, or rash of erythema multiforme type or Stevens-Johnson syndrome.
16. Have any other medical or psychological condition regarded by the Investigator as likely to interfere with subject's ability to understand and provide signed written informed consent; cooperate with study visits, tests, and procedures; and/or otherwise safely and reliably participate in the study.

### 8.3.2. Extension Period

Subjects who meet this criterion will be excluded from the Extension Period of the study:

1. Have experienced any AE during the Core Period considered by the treating Investigator or the Sponsor’s designated Medical Monitor or Responsible Medical Officer to pose a significant safety risk should study treatment be extended.

## 8.4. Subject Identification and Registration

Subjects who are candidates for enrollment into the study will be evaluated for eligibility by the Investigator to ensure that the inclusion and exclusion criteria (see Section 8.2 and Section 8.3, respectively) have been satisfied and that the subject is eligible for participation in this clinical study. The site will submit to the Sponsor an Eligibility form for each eligible subject and the Medical Monitor will confirm eligibility for all subjects prior to receipt of the first dose of AG-348.

## 8.5. Subject Randomization

Subjects who have been confirmed as eligible will be randomized in an equal ratio to a treatment arm (eg, 1:1 or 1:1:1 depending on which arms are open). The site will provide a request for randomization form (including the subject’s confirmed genotype) to the study Medical Monitor. The randomization will be stratified by PKR mutation in order to maintain balance across the dose arms for the specific mutations expected to be most frequently enrolled. The PKR mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H, and all other mutations as “other”). Since this is an open label study, randomization will not be blinded.

Please refer to the study manual for the randomization procedure.

## 8.6. Subject Withdrawal Criteria

Subjects have the right to withdraw from the study at any time for any reason. Subjects will be withdrawn from study-related procedures and treatments under any of the following conditions:

- Withdrawal of consent.
- Experiences unacceptable toxicity.
- Development of an intercurrent medical condition that precludes further participation in the trial.
- Subject requires use of a prohibited concomitant medication (Section 9.11.2.1).
- Investigator decision
- Protocol violation (nonadherence to protocol requirements).
- Pregnancy.
- Lost to follow-up.

Should a subject decide to withdraw, all efforts will be made to complete and report the protocol-defined study observations up to the time of the subject's withdrawal as completely as possible and to determine the reason for withdrawal.

In the event a subject is withdrawn from the study, the Medical Monitor must be informed. If there is a medical reason for withdrawal, the subject will remain under the supervision of the Investigator until the AE resolves, is declared chronic by the Investigator, or the subject is lost to follow-up.

When a subject withdraws from the study, the primary reason for discontinuation, if known, must be recorded in the appropriate section of the electronic case report form (eCRF) and all efforts will be made to complete and report final study observations as thoroughly as possible.

All AEs should be followed until resolution or for a period of 30 days from the last dose of study drug, whichever is shorter. Subjects with toxicity suspected to be related to study drug will continue follow-up until the AE resolves, is declared chronic by the Investigator, or the subject is lost to follow-up.

## 8.7. Replacement of Subjects

Subjects who drop out of the Core Period prior to completing the first 12 weeks of assigned dosing for reasons other than AEs may be replaced at the Sponsor's discretion.

# 9. STUDY TREATMENT

## 9.1. Description of Study Drug

AG-348 sulfate hydrate capsules will be provided as 5 mg, 25 mg, or 100 mg (free-base equivalent) of AG-348 sulfate hydrate drug substance without excipients in hard gelatin capsules.

All study drugs are for investigational use only and are to be used only within the context of this study. All study drug products will be supplied by the Sponsor. The Sponsor reserves the right to discontinue the supply of any specific capsule strength should the evolving trial experience demonstrate that the specific capsule strength fills no additional need beyond the other capsule strengths available. Please see the Investigator's Brochure for further details regarding study drug.

## 9.2. Study Drug Packaging and Labeling

AG-348 sulfate hydrate capsules are packaged in white, high-density polyethylene (HDPE) induction sealed bottles with a child-resistant screw cap.

Packaging and labeling will be prepared to meet all regulatory requirements.

## 9.3. Study Drug Storage

The recommended storage condition and expiry (where required) are stated on the product label.

All study drug products must be stored in a secure, limited-access location and may be dispensed only by the Investigator or by a member of the staff specifically authorized by the Investigator.

## 9.4. Method of Assigning Subjects to Treatment

Up to a maximum of 25 subjects will be randomized to any 1 of the dosing arms in this study. Randomization will be balanced 1:1 or 1:1:1, depending on whether 2 or potentially 3 arms are open, and will be stratified by PKR mutation (see Section 8.5). The dose and schedule of AG-348 each subject receives will be dependent upon which dose arm is open for enrollment when the subject qualifies for and is randomized into the study. Subjects in the Extension Period will continue on the dose they were randomized to in the Core Period, unless the DRT had reason to establish a different dose/schedule during the course of the Core Period (the DRT will not propose a dose higher than 360 mg BID).

## 9.5. Blinding

This is an open-label study; no blinding methods will be used.

## 9.6. Study Drug Preparation and Administration

For the initial 2 treatment arms, (Arm 1 and Arm 2) in the Core Period, AG-348 will be administered PO BID (approximately q12h, with a minimum of 10 hours between doses) over a 24-week treatment period. Starting with Day 1, dosing will be continuous; there will be no rest periods. Subjects who do not meet any of the treatment withdrawal criteria (see Section 8.6) may continue treatment for the entire 24-week treatment period.

Subjects will be dispensed the appropriate number of Sponsor-packaged, labeled bottles to allow for dosing until the next scheduled visit. The amount of study drug dispensed should be sufficient to provide an adequate reserve supply of AG-348 to ensure uninterrupted dosing in the event of an unexpected delay for the next scheduled study visit (seven extra days of dosing supply are recommended during the Core Period; 14 extra days is recommended during the Extension Period).

Subjects will be given a dosing diary to be used for each 28-day dosing period. They should record relevant information regarding their study drug in the diary (eg, confirmation that each daily dose was taken, reasons for missed doses).

Treatment compliance will be assessed based on return of unused drug and the dosing diary (see Section 9.9).

Subjects should be instructed to take their daily dose at approximately the same times each day except for dosing on in-clinic visiting days.

Subjects who undergo extensive PK/PD sampling during the Core Period (see Appendix 1.3) should be instructed from Week 3 on to bring the AM dose with them for in-clinic visits and to ingest the dose following PK/PD blood draws.

Subjects receiving limited PK/PD sampling during the Core Period (see Appendix 1.4) should be instructed to bring the AM dose with them for all in-clinic visits and to take the AM dose following PK/PD blood draws.

Subjects receiving extensive PK/PD sampling on Day 1 and 15 will also have limited PK/PD on other visit days. As a general rule, regardless of extensive or limited schedule, subjects will bring in the AM dose for all visits and take this dose following PK/PD blood draws. Subjects not continuing into the Extension Period are not required to take the Week 24/Day 169 morning dose of AG-348 after the required PK/PD blood samples are collected, as these subjects will be discontinuing the study.

A minimum of 10 hours between the AM and PM dose will be required on those dosing days. Each dose should be taken with a glass of water and consumed over as short a time as possible. AG-348 may be taken with or without food. Subjects should be instructed to swallow capsules whole and to not chew the capsules. For subjects who have difficulty swallowing tablet(s), the Medical Monitor should be contacted to discuss administration.

Subjects will receive their first dose of AG-348 in the clinic on Day 1 and then may take the remaining doses on an outpatient basis (except for in-clinic visit days, as described above).

## 9.7. Criteria for Dose Escalation, Dose Modification, or Discontinuation of Study Drug

Intra-subject dose escalations will be permitted in this study under 2 circumstances. First, the DRT may decide to re-assign subjects' doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the subjects in the terminated arm will remain in their originally assigned arm; ie, they will not count towards the enrollment quota of the arm whose dose and schedule is being adopted.

Second, a treating Investigator, with Medical Monitor approval, may elect to increase the dose for a subject in Arm 2 (50 mg BID) or a potential third arm of the study (if < 300 mg BID) to 300 mg BID if the subject is adequately tolerating his/her current dose and if their Hb has not reached at least the lower limit of the normal gender-adjusted reference range as specified by the designated central laboratory (male: 13.0 g/dL; female: 11.6 g/dL) after at least 12 weeks of treatment in the Core Period. The subject must have completed the 12-week visit during the Core Period and had all assigned tests/procedures for that visit before an intra-subject dose escalation will be allowed. An intra-subject dose escalation may also be made later than the 12-week visit in the Core Period.

Subjects in the Extension Period who have not experienced a robust and sustained increase in hemoglobin by the next scheduled visit, in particular after a dose increase, should be encouraged to discontinue AG-348 if the only benefit of continued treatment is reduction in jaundice/bilirubin levels and/or improved feeling of wellbeing.

Dosing modifications, as outlined below, will be implemented following discussions with the Medical Monitor.

Subjects should be advised not to discontinue dosing without first speaking with the treating Investigator—abrupt discontinuation of AG-348 dosing in a subject who experience a substantial increase in Hb may result in withdrawal hemolysis.

### 9.7.1. Dose Modification for Safety

The Investigator will monitor all subjects for safety and tolerability. Modification of an individual subject's dose of AG-348 will be based on AEs and observed changes in Hb levels (see Section 9.7).

**Table 1: Dose Modification for Adverse Events Deemed at Least Possibly Related to AG-348**

| Adverse Event(s) Severity[1] | AG-348 Dose Adjustment |
|---|---|
| Grade 1 | None required. |
| Grade 2 | None required; use Investigator and Medical Monitor judgment to manage, as for Grade 3 events (see below). |
| Grade 3 | Suspend dosing[2].<br>If event resolves to Grade 1 or baseline within approximately 14 days of suspension, resume dosing with at least a 1 dose-level reduction and discuss the advisability of additional dose modification with the Medical Monitor (see Table 2).<br>If event does not resolve to Grade 1 or baseline within approximately 14 days of suspension, permanently discontinue dosing, unless the benefits outweigh the risks of resuming treatment in the opinion of the Investigator as agreed upon by the Medical Monitor. |
| Grade 4 | Permanently discontinue dosing, unless the benefits outweigh the risks of resuming treatment in the opinion of the Investigator as agreed upon by the Medical Monitor. |

1. Per National Cancer Institute Common Terminology Criteria for Adverse Events.
2. A related adverse event of Grade 3 severity would generally be expected to require suspension of dosing with AG-348. However, in some circumstances in which the hemoglobin concentration has normalized prior to the onset of the related adverse event, the Investigator may elect to discuss with the Medical Monitor tapering the dose of AG-348 in order to mitigate potential withdrawal hemolysis (see Section 5.2.4.1 and Section 9.7.2).

Dose modifications for Grade 2 and 3 AEs should be maintained long enough for the Investigator to be confident that the reduced dose is being well-tolerated before considering contacting the Medical Monitor to approve re-escalation to the former dose level. Dosing for an individual subject will be discontinued permanently for Grade 3 and Grade 4 AEs that do not resolve to Grade 1 or baseline within approximately 14 days of suspension of dosing, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor.

No subjects may be re-escalated to their former dose level after a dose modification without discussion with the Medical Monitor. If, following the first dose reduction for a Grade 3 AE, the subject experiences a second occurrence of the same Grade 3 AE, then treatment with AG-348 must be immediately and permanently discontinued. However, in any subject having experienced a robust and sustained increase in Hb, AG-348 discontinuation should be done after progressive dose decrease (assuming safety considerations allow it) to avoid withdrawal hemolysis.

It should be noted that if the initial dose of 300 mg BID selected for Arm 1 demonstrates an unacceptable safety profile resulting in multiple subjects undergoing dose modifications, the DRT may exercise its option to re-assign these subjects' dose and schedule to match the dose and schedule of another study arm (eg, Arm 2 of the study, or to match the dose and schedule of a [potential] Arm 3, if implemented).

### 9.7.2. Dose Modification for Increase in Hemoglobin Level

It is presently unknown to what magnitude, how rapidly, or even whether, AG-348 will result in increased Hb levels in subjects with PK deficiency across the variety of potential PKR mutations that may be encountered during this study. Moreover, it is unknown whether any potential increases in Hb experienced by subjects in this study may be a safety risk.

As a conservative measure, this study incorporates guidance for dose modification based on potential large increases in Hb levels that exceed the midpoint of the typical normal range by gender. The intent of this guidance is to strike a balance between abruptly stopping AG-348 treatment, thereby potentially risking withdrawal hemolysis, versus reducing the dose sufficiently to allow an opportunity for Hb to settle more gradually to a level less than the midpoint of the typical normal range by gender.

The Investigator will monitor all subjects for changes in Hb levels and should adjust the dose of AG-348 as outlined in Table 2, per the following guidelines and with Medical Monitor approval.

- Males: If Hb > 15.0 g/dL and confirmed with a second test, reduce dose by at least 1 dose level (Table 2) and discuss the advisability of additional dose modification or suspension with the Medical Monitor.
- Females: If Hb > 13.5 g/dL and confirmed with a second test, reduce dose by at least 1 dose level (Table 2) and discuss the advisability of additional dose modification or suspension with the Medical Monitor.
- The treating Investigator will discuss with the Medical Monitor questions relating to additional dose modifications and the need for additional unscheduled Hb monitoring on an as needed basis.

**Table 2: Dose Reduction Table (by Dosing Arm)**

| Dosing Arm | Starting Dose | 1st Dose Reduction | 2nd Dose Reduction |
|---|---|---|---|
| Arm 1 | 300 mg BID | 200 mg BID | 100 mg BID |
| Arm 2 | 50 mg BID | 25 mg BID | TBD[1] |
| Potential Arm 3 | TBD | To approximately 50-66% of initial dose. | To approximately 25-33% of initial dose. |

1. Dose to be determined by Medical Monitor.
Abbreviations: BID = twice daily; TBD = to be determined.

Hemoglobin levels above the ULN (by gender) should be reported as AEs and graded per the CTCAE v4.03, according to the guidance provided in Section 11.2.

### 9.7.3. Individual Optimal Maintenance Dose-Finding in Extension Period

#### 9.7.3.1. Gradual Dose-taper regimen in Extension Period

All subjects in the Extension Period who are receiving doses > 25 mg BID will undergo an individual and gradual dose-taper regimen to identify their individual optimal maintenance doses, defined as the dose that results in ≤ 1.0 g/dL decrease in hemoglobin compared to the pretaper Hb value on at least 2 weekly measurements following each dose taper step. The minimum dose target is 25 mg BID.

Table 3 shows the various dose levels to be used. Other dose levels should not be used during the dose taper.

**Table 3: Gradual Dose-Taper Regimen (by Actual Dose)**

| Starting Dose | 1st Dose Reduction | 2nd Dose Reduction | 3rd Dose Reduction | 4th Dose Reduction |
|---|---|---|---|---|
| 300 mg BID | 200 mg BID | 100 mg BID | 50 mg BID | 25 mg BID |
| 200 mg BID | 100 mg BID | 50 mg BID | 25 mg BID | N/A |
| 100 mg BID | 50 mg BID | 25 mg BID | N/A | N/A |
| 50 mg BID | 25 mg BID | N/A | N/A | N/A |

Abbreviations: BID = twice daily; N/A = not applicable.

After each dose reduction, the subject will remain on the reduced dose for a period of 3 weeks. Hb will be measured 1 week and 2 weeks after the start of the 1st reduced dose. If Hb (mean of these 2 Hb levels) has decreased by ≤ 1.0 g/dL from the pretaper value, the dose should be decreased to the next level at the 3 week visit, and the process repeated. If, at any step during this process, Hb (mean of levels after 1 week and 2 weeks at a reduced dose) has decreased by > 1.0 g/dL below the pretaper Hb value, the subject's dose should be increased to the next higher dose level.

Table 4 shows the Schedule of Assessments during the dose-taper regimen. The Hb values used to guide dose taper decisions (pre-dose taper, after 1 and 2 weeks of dose taper at each step) should be those provided by the central laboratory. In exceptional cases when these central laboratory Hb values are not available at the visit 3 weeks after the start of a tapering step, Hb values from the local laboratory may be used, provided that the pre-dose taper value from the same local laboratory is available for comparison.

Blood sampling for weekly Hb monitoring during the gradual dose-taper regimen may be performed at the subject's home by a visiting nurse, at the local physician's office, or at a local laboratory, with the samples being shipped promptly to the central laboratory.

## Table 4: Schedule of Assessments for Gradual Dose-Taper Regimen in the Extension Period[1]

| | 1st dose reduction | | | 2nd dose reduction (if applicable) | | | 3rd dose reduction (if applicable) | | | 4th dose reduction (if applicable) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study day** | Next scheduled visit in Extension Period | 7 days after 1st dose reduction[2] | 14 days after 1st dose reduction[2] | 21 days after 1st dose reduction | 7 days after 2nd dose reduction[2] | 14 days after 2nd dose reduction[2] | 21 days 2nd dose reduction | 7 days after 3rd dose reduction[2] | 14 days after 3rd dose reduction[2] | 21 days after 3rd dose reduction | 7 days after 4th dose reduction[2] | 14 days after 4th dose reduction[2] | 21 days after 4th dose reduction |
| **Visit window** | ± 2 weeks | ± 2 days | ± 2 days | ± 2 days | ± 2 days | ± 2 days | ± 2 days | ± 2 days | ± 2 days | ± 2 days | ± 2 days | ± 2 days | ± 2 days |
| **Procedure** | | | | | | | | | | | | | |
| Dose reduction | X | | | X | | | X | | | X | | | |
| Hematology/ haptoglobin[3] | | X | X | X | X | X | X | X | X | X | X | X | |

1. All subjects in the Extension Period will undergo a gradual dose-taper regimen starting at the next scheduled clinic visit, as per Table 3 in Section 9.7.3.1. Follow the Schedule of Assessments for the appropriate assessments to be conducted along with the 1st dose reduction visit.

2. For greater subject convenience, blood samples at the time points displayed in shaded columns may be performed at home by qualified study personnel (eg, home health care nurse), at the subject's local physician office, or at a local lab, with the samples being shipped promptly to the central lab.

3. Hematology parameters include haptoglobin and complete blood count (CBC), with the latter to include hematocrit, hemoglobin (Hb), red blood cell count, absolute reticulocyte count, percent reticulocyte count, mean corpuscular volume, mean corpuscular Hb, mean corpuscular Hb concentration, red cell distribution width, nucleated red blood cell count, white blood cell count with differential, absolute neutrophil count, absolute lymphocyte count, and platelet count.

### 9.7.4. Stopping Criteria

Dosing for an individual subject will be discontinued permanently for Grade 3 AEs that do not resolve to Grade 1 or baseline within approximately 14 days of suspension of dosing, and for Grade 4 AEs, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor (Section 9.7.1). Other reasons for treatment termination are provided in Section 8.6.

## 9.8. Duration of Subject Participation

The duration of treatment for all subjects will be up to 24 weeks in the Core Period. Subjects who safely tolerate AG-348 and for whom the Investigator agrees with continuation of AG-348 treatment may be eligible to immediately roll over to the Extension Period for continued treatment (up to 2 years following completion of the Core Period).

## 9.9. Treatment Compliance

During in-clinic visits, doses of AG-348 will be ingested by the subject under the supervision of clinical facility personnel. For at-home dosing, subjects will be given a dosing diary to be used for the duration of the 24-week Core Period; the diary will also be used by subjects who roll over to the Extension Period. Subjects should record relevant information regarding their study drug in the diary (eg, confirmation that each daily dose was taken, reasons for missed doses) and return the diary at each study visit.

## 9.10. Study Drug Accountability

Accountability for the study drug at the clinical facility is the responsibility of the Investigator. The Investigator will ensure that the study drug is used only in accordance with this protocol. Where allowed, the Investigator may choose to assign drug accountability responsibilities to a pharmacist or other appropriate individual.

The Investigator or delegate will maintain accurate drug accountability records indicating the drug's delivery date to the site, inventory at the site, use by each subject, and return to the Sponsor or its designee (or disposal of the drug, if approved by the Sponsor). These records will adequately document that the subjects were provided the doses as specified in the protocol and should reconcile all study drug received from the Sponsor. Accountability records will include dates, quantities, batch/serial numbers, expiration dates (if applicable), and subject numbers. A monitor will review drug accountability at the site on a schedule agreed to by the Sponsor.

Study drug must not be used for any purpose other than the present study.

All unused and used study drug will be retained at the site until it is inventoried by the Study Monitor after database lock has occurred. All used, unused or expired study drug will be returned to the Sponsor or its designee or, if authorized, disposed of at the study site per the site's Standard Operating Procedures and documented. All material containing AG-348 will be treated and disposed of as hazardous waste in accordance with governing regulations.

## 9.11. Prior and Concomitant Medications and Treatments

### 9.11.1. Prior Medications and Procedures

All medications administered and procedures conducted within 28 days prior to, or from Screening Visit #1 until the first day of study drug administration, whichever interval is longer, are to be recorded on the source documentation and included in the eCRF.

### 9.11.2. Concomitant Therapy

All concomitant medications and procedures administered from 28 days prior to, or from Screening Visit #1 until, the first day of study drug administration (whichever interval is longer) through the last Follow-up Visit must be recorded in the appropriate section of the source documentation and eCRF, along with dosage information, dates of administration, and reason for use.

Investigational drugs must be discontinued no less than 28 days prior to the first dose of study drug.

In vitro studies using human liver microsomes and recombinant CYP enzymes have shown that AG-348 is primarily metabolized by CYP3A4 (>70%), with minor contributions from CYP2C9, CYP2C8, and CYP1A2. In addition, AG-348 has been shown to be a weak time-dependent CYP3A4 inhibitor and a potential inducer of CYP3A4 and CYP2B6 in vitro. In vitro transporter studies have shown that AG-348 is a substrate and inhibitor of P-gp. Based on these results, below is a list of concomitant therapy to be avoided and concomitant therapy requiring careful monitoring.

Since AG-348 exhibits pH-dependent solubility, proton-pump inhibitors and H2-receptor antagonists may decrease the absorption of AG-348.

#### 9.11.2.1. Concomitant Therapy to be Avoided (Prohibited)

The following therapies are contraindicated during this study:

- Strong inhibitors of CYP3A4 (listed in Appendix 4.1).
- Products known to inhibit CYP3A4, such as grapefruit or grapefruit juice.
- Strong inducers of CYP3A4 (listed in Appendix 4.2).
- Strong inhibitors of P-gp (listed in Appendix 4.3).
- Digoxin, a P-gp transporter-sensitive substrate.

If a subject is taking any medication listed in Appendices 4.1, 4.2, or 4.3 and/or digoxin prior to enrolling in the study, the medication must be discontinued within 5 days prior to Day 1 dosing.

- Hematopoietic stimulating agents (eg, EPOs, granulocyte colony stimulating factors, thrombopoietins) must be discontinued no fewer than 28 days prior to the first dose of study drug. (Folic acid 1 mg PO per day is required for all subjects. B12 injections are permitted for subjects with a prior diagnosis of B12 deficiency syndromes, but must be repleted to stability of the Hb and mean corpuscular volume [MCV] prior to enrollment in the study).

- Anabolic steroids, including testosterone preparations, administered for anemia must be discontinued no less than 28 days prior to the first dose of study drug.
- As the target population for this study consists of transfusion independent subjects and transfusion of blood products could confound key endpoints of the study, blood transfusions of any type must be strictly avoided except in cases of compelling medical need. If medical circumstances permit, the Medical Monitor should be contacted for discussion before any transfusions are administered.

#### 9.11.2.2. Concomitant Therapy Requiring Careful Monitoring (Use with Caution)

The following therapies should be avoided and replaced with alternative treatments. If this is not possible, subjects receiving these drugs should be adequately monitored.

- Corticosteroids (sensitive substrates of CYP3A4 and weak CYP3A4 inducers).
- Sensitive substrates of CYP3A4 (listed in Appendix 4.4).
- Moderate inhibitors of CYP3A4 (listed in Appendix 4.1).
- Sensitive substrates of CYP2B6 (listed in Appendix 4.5).
- Proton-pump inhibitors and H2-receptor antagonists (listed in Appendix 4.6) should be used with caution. Antacids such as magnesium hydroxide and aluminum hydroxide can be used with AG-348.
- AG-348, being a potential CYP3A4 inducer, has the potential to reduce the effectiveness of oral contraceptives. Therefore, women using oral contraceptives must also utilize a barrier method while enrolled in the study and till at least 30 days after their last dose of study drug, as specified in Inclusion Criterion 12 (see Section 8.2).
- Drugs that displace unconjugated bilirubin from albumin (including some common sulfa antibiotics [including sulfamethoxazole/trimethoprim], cephalosporins, salicylates, and aminophylline) should be used with caution with the understanding that subjects with elevated levels of unconjugated bilirubin may potentially be at risk for kernicterus syndrome (Strauss, et al. 2006).

#### 9.11.2.3. Allowed Concomitant Therapy

Medications and treatments other than those specified above are permitted during the study. All intercurrent medical conditions will be treated at the discretion of the Investigator according to acceptable local standards of medical care. The expected subject comedications deferoxamine, deferasirox, deferiprone, and oral penicillin are not expected to interact with AG-348. Subjects may receive analgesics, anti-emetics, anti-infectives (including penicillins), and antipyretics as medically indicated and consistent with the guidance in the previous 2 sections. Subjects must continue taking at least 1 mg of folic acid for the duration of the study.

All concomitant medications, including transfusions of blood products, procedures performed during the study, including those used to treat AEs, will be recorded on the eCRF.

## 9.12. Management of Nausea, Vomiting, and Diarrhea

As the primary objective of this study is to assess the safety and tolerability of up to 24 weeks of AG-348, routine use of prophylactic anti-emetic and antidiarrheal or other classes of medications is prohibited. However, if subjects experience AEs relating to nausea, vomiting, or diarrhea, these may be treated at the Investigator's clinical discretion with recommended medications as follows:

- Nausea/vomiting: allowed agents include standard clinical dosing with palonosetron (Aloxi), dolasetron (Anzemet), or promethazine (Phenergen). Not recommended are aprepitant (Emend; CYP3A4 inhibitor), ondansetron (Zofran; CYP3A4 inducer), chlorpromazine (Thorazine; CYP3A4 inducer), prochlorperazine (Compazine; CYP3A4 substrate, QT prolongation has been reported), and granisetron (Kytril; CYP3A4 substrate, QT prolongation has been reported).
- Diarrhea: recommended management includes standard clinical dosing with Kaopectate or other nonabsorbable anti-diarrheals, diphenoxylate/atropine (Lomotil), or loperamide (Imodium). Loperamide is the least preferred choice because it is both a substrate and inhibitor for CYP3A4, a substrate for CYP2B6, and a substrate for P-gp.
- For the use of any medications not specifically mentioned above the Investigator may confer with the Sponsor’s Medical Monitor.

# 10. STUDY ASSESSMENTS

## 10.1. Schedules of Assessments

The Schedules of Assessments for this study are provided in Appendix 1.

After obtaining written informed consent, subjects will undergo screening evaluations. The Screening visit is to be conducted within approximately 42 days prior to first dose of study treatment.

During the Core Period, subjects will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12) and monthly through Week 24 (Weeks 16, 20, and 24). Subjects who safely tolerate AG-348 through Week 24 (Core Period) and for whom the Investigator agrees with continuation of AG-348 treatment may be eligible to immediately enter the Extension Period for continued treatment upon agreement of the treating Investigator and the Medical Monitor or Responsible Medical Officer. Study visits for safety and clinical activity assessments will occur approximately every 3 months during the Extension Period. All subjects will undergo a follow-up assessment 4 weeks after the last dose of AG-348, regardless of whether this was due to discontinuation, the last dose in the Core Period for a subject who chooses not to continue in the Extension Period, or the last dose of the Extension Period, except for those who may continue treatment in an extension study (to be designed) after having completed the Extension Period.

Although *not* encouraged, as a convenience for subjects who travel long distances to the study site, in-clinic visits on Day 8 and Day 22 may be performed by the subject's primary care physician if necessary and must be approved by the Sponsor on a case-by-case basis. For details, please refer to Appendix 1.1. For subjects having their Day 8 and/or Day 22 visits performed by the primary care physician, the principal investigator will him or herself, or have a qualified research nurse or other designated site staff member, make telephone contact with the subject to inquire about any AEs. These must be recorded as if the subject appeared in the main study center. The Principal Investigator will exercise prudent clinical judgment in determining any clinical course of action to take based on any AEs discovered. These telephone contacts on Day 8 and Day 22 must be explained to the subject in advance and scheduled in advance to maximize the likelihood of successfully making contact.

Having in-clinic visits on Days 8 and/or Day 22 performed by the primary care physician will necessitate re-scheduling certain assessments that the primary care physician's office may not be reasonably expected to perform. Table 5 summarizes the details of the re-scheduling of these assessments as described in Appendix 1.1.

During the Extension Period, all scheduled visits, with the exception of blood draws for those subjects undergoing gradual dose-taper regimen, must be conducted by the Investigator and at the participating authorized investigative site; local primary care visits will not be allowed.

**Table 5: Summary of Assessments when Day 8 and/or Day 22 In-Clinic Visits are Performed by Primary Care Physician (Core Period)**

| Day 8 Visit by Primary Care Physician | | | | Day 22 Visit by Primary Care Physician | | | |
|---|---|---|---|---|---|---|---|
| Primary care office | Main study site | Assessment to move to Day 15 (main study site) | Assessments not required | Primary care office | Main study site | Assessment to move to Day 43 (main study site) | Assessments not required |
| Hematology to central laboratory | Phone contact with subject | 12-lead ECG | VS; serum chemistry | Hematology sample to central laboratory | Phone contact with subject | 12-lead ECG | VS; serum chemistry; coagulation; haptoglobin; EPO level; carboxy-hemoglobin; PK/PD |

Abbreviations: ECG = electrocardiogram; EPO = erythropoietin; PK/PD = pharmacokinetics/pharmacodynamics; VS = vital signs.

Whenever more than 1 assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (eg, VS first, ECG, blood draw). The timing of these assessments should allow the PK blood draw to occur at the exact nominal time. The order of procedures may be revised with prior discussion between Sponsor and Site.

Minor adjustments to the timing, number of planned safety monitoring procedures (eg, VS, ECG, blood draw), and PK/PD assessments may be made during the course of the study based on collected data to ensure appropriate safety monitoring and will not require a protocol amendment. These minor changes will require prior approval from the Sponsor's Medical Monitor (or Responsible Medical Officer) as well as appropriate documentation in the study records. The addition of new safety monitoring procedures or other assessments will require a protocol amendment.

## 10.2. Informed Consent and Confirmation of Eligibility

A complete description of the study is to be presented to each potential subject and a signed and dated informed consent is to be obtained before any study specific procedures are performed. Separate informed consent forms will be used for the Core and Extension Periods.

Subject's eligibility will be confirmed at Screening and within 24 hours prior to study treatment dose. If a subject is determined to be ineligible, the subject will be excluded from participation.

## 10.3. Demographic Data, Medical and Medication History

Subject demographic data, including gender, date of birth, age, race, and ethnicity, will be obtained at Screening. Collection of demographic data will be modified by country regulatory requirements, as appropriate.

Medication history, including all relevant prior medical history and current medical conditions, will be obtained at the Screening assessment and on Day -1.

All concomitant medications administered and procedures conducted within 28 days prior to, or from Screening Visit #1 until the first day of study drug administration, whichever interval is longer, should be reported in the source documentation and eCRF.

Investigators will be asked to provide information on the subject's history of any medical diagnoses (eg, iron overload) and surgical procedures (eg, splenectomy, cholecystectomy) pertaining to their diagnosis of PK deficiency and prior available CBCs over the preceding 6 months and transfusion history over the preceding 12 months prior to the date of signing informed consent.

## 10.4. PKR Enzymatic Assay and PKR Genotyping

Assessments for PKR enzymatic activity and PKR genotyping will be performed at Screening only for confirmation of study eligibility. PKR enzymatic assays will be conducted at [redacted] or any participating investigative site's local hematology laboratory. PKR genotyping will be conducted at [redacted].

## 10.5. Safety Assessments

### 10.5.1. Physical Examination, Height, and Weight

A complete physical examination (including neurological examination; genital and rectal examinations will be performed at the discretion of the Investigator) will be obtained at Screening, Baseline, Week 12, and the Follow up Visit (Week 28). The neurological examination must include an assessment of general orientation and mental status including level of alertness (assess as normal or abnormal and specify any abnormality).

Limited, focused physical examinations will be performed at all other visits during the Core Period. Focused physical examinations, including neurological examination, will continue every 3 months during the Extension Period; a complete physical examination will be performed at Month 30.

Any findings will be recorded on the eCRF.

Height will be collected at Screening only.

### 10.5.2. Vital Signs

Vital signs, including systolic and diastolic BP, heart rate, respiratory rate, and temperature, will be obtained according the Schedules of Assessments (Appendix 1).

Vital sign measurements will be repeated if clinically significant or machine/equipment errors occur. Out of range BP or heart rate measurements will be repeated at the Investigator's discretion. Any confirmed, clinically significant vital sign measurements will be recorded as AEs.

### 10.5.3. Electrocardiogram

A 12-lead ECG will be obtained according to the Schedules of Assessments (Appendix 1). The ECGs will be measured using an ECG machine that reports the heart rate and the portion of the ECG wave from the beginning of the P wave to the beginning of the QRS complex (PR), QRS,

QT, QTcB (Bazett correction formula; may also be calculated), and QTcF intervals (may also be calculated). Only QTcF (not QTcB) will be used for determination of eligibility.

The 12-lead ECGs should be obtained following 5 minutes of recumbency. ECGs will be repeated if clinically significant abnormalities are observed, if artifacts are present, or if machine/equipment errors occur. Any confirmed, clinically significant ECG findings will be recorded as AEs.

### 10.5.4. DXA Scans

DXA scans (hip and spine) will be performed at Screening to obtain T and Z scores and bone mineral density that will serve as a baseline measure for all enrolling subjects. An additional DXA scan for the Core Period will be conducted in the interval between Week 24 and Week 28, and scans will be conducted at Months 12, 18, 24, and 30 during the Extension Period as indicated in the Schedules of Assessments (Appendix 1.2). All redacted DXA scan reports must be held at the study site and will be collected by the Sponsor.

### 10.5.5. Safety Laboratory Assessments

#### 10.5.5.1. Hematology, Serum Chemistry, Coagulation Studies, and Urinalysis

Laboratory values obtained prior to Screening and RBC antibodies obtained at Screening will be performed at a local laboratory. On-study clinical laboratory evaluations are to be performed by a central laboratory. If Investigators believe that it is clinically indicated to obtain safety laboratory results from their own local laboratories on the day of the subject's visit, and before the results are returned from the designated central laboratory, they are free to exercise their discretion to do so. Investigators should be aware that since the Hb level is a secondary endpoint of the study, it behooves them to minimize blood volumes drawn, and samples for the central lab must still be collected, since it will serve as the official lab result for this study.

Clinical laboratory evaluations are to be collected according to the Schedules of Assessments (Appendix 1). In addition, all clinically significant laboratory abnormalities noted on testing will be followed by repeat testing and further investigated according to the judgment of the Investigator. Please note that serum estradiol, free and total testosterone, and CBC will be collected in the AM at any 2 time points during Screening at least 2 days apart in addition to Baseline/Day 1 (total of 3 time points prior to Day 1 dosing).

The safety laboratory parameters listed below are to be determined.

| | |
|---|---|
| **Hematology**: | HCT, Hb, RBC count, absolute reticulocyte count, percent reticulocyte count, MCV, mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count with automated (or manual, as indicated) differential, ANC, and absolute lymphocyte count (ALC), and platelet count.<br><br>In the event that the designated central laboratory for the study is unable to provide a valid result for any specific component of the defined CBC for a specific subject, the site may be asked to have the test performed at their local laboratory. The result of the local CBC will be entered into the study database along with the local normal reference range.<br><br>G6PD (may be conducted at Mayo Medical Laboratories [Rochester, MN] or any participating investigative site's local hematology laboratory) and RBC antibody screen will be performed at Screening only. |
| **Other** | EPO, Hp, COHb, hepcidin, 25-hydroxy vitamin D2 and D3. |
| **Serum Chemistry:** | Alkaline phosphatase (ALP), sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide ($CO_2$) or bicarbonate, albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, LDH, ALT, AST, total bilirubin, indirect bilirubin (and estimated creatinine clearance or glomerular filtration rate for Screening only, as appropriate). |
| **Sex Hormones**: | Testosterone (total and free), estrone, and estradiol. FSH will only be performed at Screening for female subjects only for confirmation of postmenopausal status. |
| **Bone Turnover:** | Serum osteocalcin-N-mid and CTX. |
| **Lipids:** | Total cholesterol, HDL-C, triglycerides. |
| **Iron Panel:** | Iron, total iron-binding capacity (TIBC), transferrin saturation, ferritin. |
| **Coagulation Studies:** | Fibrinogen, aPTT, INR. |
| **Urinalysis**: | Color and appearance, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, pregnancy screen (dipstick), occult blood. Microscopic inspection of sediment will only be performed for cause or to investigate an abnormal dipstick finding per the Investigator's discretion. |

#### 10.5.5.2. Screening Serology

A blood sample for serology, including HBsAg, HCV Ab screen, and HIV1 and HIV2 Ab, is to be collected from all subjects at Screening.

### 10.5.6. Menstrual Cycle Diary

Menstruating female subjects will be required to fill out a paper-based menstrual cycle diary each month in order to monitor any changes. Diaries will be dispensed and collected as indicated in the Schedules of Assessments (Appendix 1). Subjects will record the start date, stop date, and any notable characteristics of each menstrual cycle.

### 10.5.7. Adverse Events

Each subject will be carefully monitored for the development of any AEs, including AESIs (Section 11.1.5), throughout the study, from signing of the informed consent through all scheduled study follow-up visits, or withdrawal of consent, whichever occurs first. In addition, SAEs (Section 11.1.4) that are assessed as possibly or probably related to study treatment that occur > 30 days post-treatment also are to be reported.

Adverse events will be evaluated by the Investigator and recorded as described in the Schedules of Assessments. On dosing visits, all AEs (elicited and spontaneously reported) will be continuously evaluated by the Investigator and recorded. At any nondosing day visit, AEs will be evaluated by the Investigator and recorded.

Any AEs already documented at a previous assessment and designated as ongoing will be reviewed at subsequent visits or assessment time points as necessary. If these AEs have resolved, this will be documented.

All AEs will be graded using the CTCAE v4.03 grading system (Appendix 3).

Complete details of AE monitoring are provided in Section 11.

## 10.6. Pharmacokinetic Assessments

The first approximately 10 subjects treated in the Core Period, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 1.3. The remainder of treated subjects will undergo limited PK sampling as detailed in Appendix 1.4.

The in-clinic visit on Day 22 may be performed by the subject's primary care physician if necessary and must be approved by the Sponsor on a case by case basis. In this instance, PK sampling will not be required on Day 22. (Additional details regarding Day 8 and Day 22 visits performed by the subject's primary care physician can be found in Table 5.)

During the Extension Period, predose PK samples will be drawn for the measurement of trough levels of AG-348 and AGI-8702 at each study visit (every 3 months; see Appendix 1.2).

The collection times for postdose PK samples will start from the time that dosing is completed; eg, a PK draw at 30 minutes will be collected 30 minutes after the last capsule has been ingested. The completion time of each dose will be recorded.

Procedures for sample collection and processing will be provided in a separate study manual. The actual time point of each sample collection will be recorded.

Samples for PK and PD assessments may be retained for up to 2 years from collection.

## 10.7. Pharmacodynamic Assessments

The first approximately 10 subjects treated in the Core Period, contingent on clinical site feasibility, will undergo extensive PD sampling for 2,3-DPG and ATP as detailed in Appendix 1.3. The remainder of treated subjects will undergo limited PD for 2,3-DPG and ATP sampling as detailed in Appendix 1.4. During the Extension Period, predose PD samples will be drawn for the measurement of trough levels of 2,3-DPG, ATP, [redacted] at each study visit (every 3 months; see Appendix 1.2).

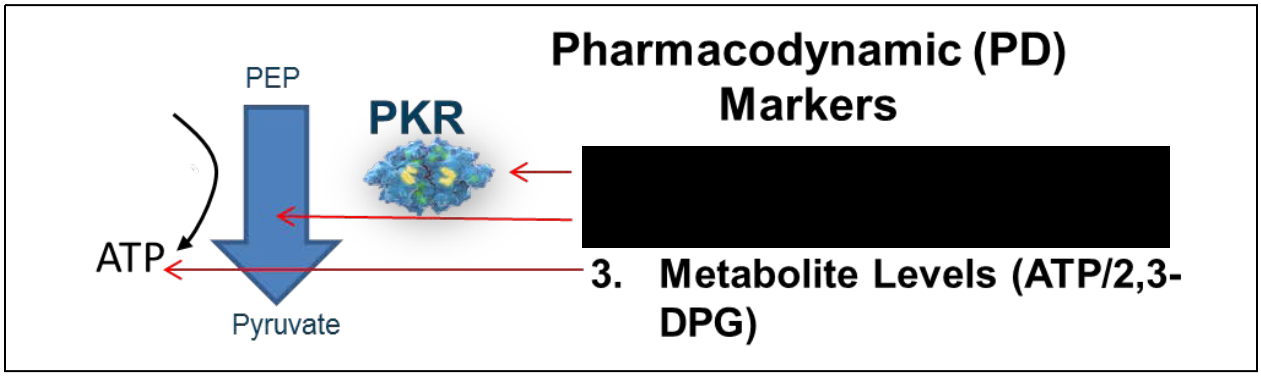
The collection times for postdose PD samples will start from the time that dosing is completed; eg, a PD draw at 30 minutes will be collected 30 minutes after the last capsule has been ingested. The completion time of each dose will be recorded.

Procedures for sample collection and processing will be provided in a separate study manual. The actual time point of each sample collection will be recorded.

The in-clinic visit on Day 22 may be performed by the subject's primary care physician if necessary and must be approved by the Sponsor on a case by case basis. In this instance, PD sampling will not be required on Day 22. (Additional details regarding Day 8 and Day 22 visits performed by the subject's primary care physician are provided in Table 5.)

Figure 3 provides a brief schematic outlining the PKR reaction and how each of these PD assessments fits into a complete mechanistic understanding of the action of AG-348.

**Figure 3: PKR Enzymatic Reaction**




Abbreviations: 2,3-DPG = 2,3 diphosphoglycerate; ATP = adenosine triphosphate; PEP = phosphoenolpyruvate; PKR = pyruvate kinase isoform R.

The PKR enzyme catalyzes the PEP to pyruvate reaction, with concomitant formation of ATP.

- Binding of AG-348 to the PKR tetramer can be assessed through an ex vivo biochemical assay of cell lysates from AG-348 treated subjects. Because WBCs contain a high level of pyruvate kinase from a nonPKR pyruvate kinase isoform, WBCs are first removed by filtration before the purified red cells are frozen.

[redacted]

[redacted] It has been reported in the literature that there may be compensatory expression of PKM2 in the RBCs of some subjects with PKR deficiency (Black, et al. 1979; Kahn, et al. 1975; Rijksen, et al. 1990). Therefore, levels of PKM2 and appropriate reference proteins (eg actin, GAPDH) may be evaluated in these whole blood samples.

- [redacted]
- AG-348 target engagement [redacted] activity has been shown in preclinical models and healthy adult subject clinical studies to result in accumulation of ATP and depletion of the upstream metabolite 2,3-DPG. Therefore, levels of these metabolites will be measured by mass spectrometry in frozen whole blood samples.

Blood samples will be stored at the site and regularly transported at -80°C (±10 °C) to the bioanalytical laboratory for analysis. Procedures for sample collection and processing will be provided in a separate study manual.

## 10.8. Ordering of Blood Sample Collection

When more than 1 blood sample is collected at the same nominal time, the samples will be collected in the following order:

1. Safety laboratory assessments.
2. PD (2,3 DPG, ATP).

[redacted]

4. PK.

[redacted]

## 10.9. Sample Processing, Storage, and Shipment

Instructions for the processing, storage and shipment of all study samples for central analysis will be provided in a separate study manual.

# 11. ADVERSE EVENTS

Monitoring of AEs, including SAEs, AESIs, and AEs leading to discontinuation, will be conducted throughout the study. Adverse events and SAEs will be recorded in the source documentation and eCRF from time of the signing informed consent through the Follow-up Visits for randomized subjects. All AEs should be monitored until they are resolved or are clearly determined to be due to a subject's stable or chronic condition or intercurrent illness(es).

All SAEs will be followed through 30 days after the last dose of study treatment or until the SAE has resolved. Any SAEs that are assessed as possibly or probably related to study treatment that occur > 30 days post-treatment also are to be reported.

## 11.1. Definition of Adverse Events

### 11.1.1. Adverse Event

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered study drug-related. An AE (also referred to as an adverse experience) can be any unfavorable and unintended sign (eg, an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An AE can arise from any use of the drug (eg, off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

### 11.1.2. Suspected Adverse Reaction

A suspected adverse reaction is any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of expedited safety reporting, 'reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the AE.

### 11.1.3. Unexpected Adverse Event

An unexpected AE is one for which the nature or severity of the event is not consistent with the applicable product information, eg, the Investigator's Brochure.

### 11.1.4. Serious Adverse Event

An AE or suspected adverse reaction is considered serious (SAE) if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death.
- Life-threatening (meaning that the subject was at immediate risk of death from the reaction as it occurred; ie, it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form).
- Inpatient hospitalization or prolongation of existing hospitalization. Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected manner during the study (eg, surgery performed earlier than planned).
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

- Congenital anomaly/birth defect.
- Important medical event. An important medical event is an event that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the subject or subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in the definitions for SAEs. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

### 11.1.5. Adverse Events of Special Interest

An AESI can be serious or nonserious. Ongoing monitoring and rapid communication (within 24 hours) by the Investigator to the Sponsor is required to allow for further characterization and reporting to regulatory authorities.

#### 11.1.5.1. Transaminase Increase

Transaminase increase is an AESI for AG-348. Transaminase increases of > 2.5 × baseline or those that have increased to ≥ Grade 2 in severity should be reported as an AESI within 24 hours. The increase should subsequently be investigated as the Investigator deems appropriate.

Transaminase levels should then be monitored weekly until they have decreased to < 2.5 × baseline. If a dose adjustment is being considered, the Investigator should consult with the Medical Monitor and refer to Section 9.7.1.

In the event of a transaminase increase of > 2.5 × baseline or 1 that has increased to ≥ Grade 2 in severity, the study site should report this occurrence to the Sponsor using the SAE form within 24 hours of their first knowledge of the event.

## 11.2. Procedures for Reporting Adverse Events, Adverse Events of Special Interest, and Serious Adverse Events

Each subject must be carefully monitored for the development of any AEs. This information should be obtained in the form of nonleading questions (eg, “How are you feeling?”) and from signs and symptoms detected during each examination, observations of study personnel, and spontaneous reports from subjects.

All AEs (serious and nonserious) spontaneously reported by the subject and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures will be recorded in the source documentation and eCRF. Any clinically relevant deterioration in laboratory assessments or other clinical findings is considered an AE and must be recorded in the appropriate source documentation and eCRF.

Treatment-emergent abnormal clinical laboratory results should generally be reported as AEs if there are accompanying symptoms; if additional diagnostic evaluations or medical (including drug therapy) or surgical interventions are undertaken; if a change in study drug dosing or study drug discontinuation is required; or, if the laboratory result is considered clinically significant by the Investigator.

Although it is an objective of this study to determine if treatment with AG-348 results in increased Hb levels in subjects with PK deficiency, overshoot of the Hb level above the ULN by gender is not recommended and should be reported as an AE, graded per the CTCAE v4.03. When possible, signs and symptoms indicating a common underlying pathology should be noted as 1 comprehensive event.

All AESIs and SAEs that occur during the course of the study must be promptly reported by the Investigator to [redacted] Global Safety and Pharmacovigilance (see contact information below). Any deaths and any AEs assessed as life-threatening are to be reported immediately. Any AESIs and any SAEs that are not life-threatening/do not result in death are to be reported within 24 hours from the point in time when the Investigator first becomes aware of the event. All AESI and SAEs must be reported, regardless of whether they are considered causally related to AG-348.

For each AESI or SAE, an SAE form is to be completed, to include subject number, a narrative description of the event, and an assessment by the Investigator as to the severity of the event and its relatedness to study drug. Follow-up information about the event may be requested by the Sponsor or Medical Monitor.

**Contact Information:**

[redacted] **Global Safety and Pharmacovigilance**

SAE Hotline (United States): [redacted]
SAE Fax (United States): [redacted]

SAE Hotline (France): [redacted]
SAE Fax (France): [redacted]

SAE Hotline (Netherlands): [redacted]
SAE Fax (Netherlands): [redacted]

SAE Hotline (Italy): [redacted]
SAE Fax (Italy): [redacted]

SAE Hotline (United Kingdom): [redacted]
SAE Fax (United Kingdom): [redacted]

Email: [redacted]

If there are serious, unexpected adverse drug reactions associated with the use of AG-348, the Sponsor will notify the appropriate regulatory agency(ies) and all participating Investigators on an expedited basis. The local IRB/IEC will be promptly notified based on local regulations where required by the IRB/IEC of all serious, unexpected adverse drug reactions involving risk to human subjects.

All AEs, whether serious or not, will be described in the source documents in the database. All new events, as well as those that worsen in intensity or frequency relative to baseline, which occur after signing the informed consent through the final Follow-up Visit (Day 29 ± 3 days) must be recorded. Adverse events that are ongoing at the time of treatment discontinuation should be followed up to 30 days after the last dose of study treatment. All SAEs will be followed up to 30 days after the last dose of study treatment, or until the SAE has resolved.

Information to be reported in the description of each AE includes:

- A medical diagnosis of the event (if a medical diagnosis cannot be determined, a description of each sign or symptom characterizing the event should be recorded).
- The date of onset of the event.
- The date of resolution of the event.
- Whether the event is serious or not.
- Intensity of the event (see below for definitions).
- Relationship of the event to study treatment (see below for definitions).
- Action taken: none; change in the study drug administration (eg, temporary interruption in dosing); drug treatment required; nondrug treatment required; hospitalization or prolongation of hospitalization required (complete SAE page); diagnostic procedure performed; subject discontinued from the study (complete End of Study Visit).
- Outcome: subject recovered without sequelae; subject recovered with sequelae; event ongoing; subject died (notify the Medical Monitor immediately, and complete the SAE form).

**Intensity** of all AEs will be graded according to CTCAE v4.03 (Appendix 3).

**Relationship** to study drug administration will be determined by the Investigator according to the following criteria:

- Not Related: Exposure to the study treatment did not occur, or the occurrence of the AE is not reasonably related in time, or the AE is considered unlikely to be related to the study treatment.
- Possibly Related: The study treatment and the AE were reasonably related in time, and the AE could be explained equally well by causes other than exposure to the study treatment. An attribution of possibly related means that there are facts in evidence to suggest a possible relationship.
- Probably Related: The study treatment and the AE were reasonably related in time, and the AE was more likely explained by exposure to the study treatment than by other causes, or the study treatment was the most likely cause of the AE. An attribution of probably related means that there are facts in evidence to suggest a probable relationship.

For the purpose of safety analyses related to final database review, all AEs that are classified as Possibly Related or Probably Related will be considered treatment-related AEs.

## 11.3. Pregnancy Reporting

Pregnancy is neither an AE nor an SAE, unless a complication relating to the pregnancy occurs (eg, spontaneous abortion, which may qualify as an SAE). However, any pregnancy in a participating female subject or a female partner of a participating male subject that occurs during this study or within 30 days following the last dose of AG-348 must be reported to the Sponsor or Medical Monitor within 30 days of being notified of the pregnancy.

The Investigator must follow up and document the course and outcome of all pregnancies even if the subject was discontinued from the study or if the study has finished. The female subject or partner of a male subject should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus. Monitoring should continue until conclusion of the pregnancy.

All outcomes of pregnancy must be reported by the Investigator to the Sponsor or Sponsor's designee on a Pregnancy Outcome Report form within 30 days after he/she has gained knowledge of the delivery or elective abortion.

Any SAE that occurs during pregnancy must be recorded on the SAE report form (eg, maternal serious complications, spontaneous or therapeutic abortion, ectopic pregnancy, stillbirth, neonatal death, congenital anomaly, or birth defect) and reported within 24 hours in accordance with the procedure for reporting SAEs.

All subjects, male and female, must agree to use effective contraception during the entire study and for 30 days following the last dose of AG-348. Abstinence is an acceptable method only when this is in line with the normal life style of the subject, meaning that the subject plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence (eg, calendar, symptothermal, and postovulation methods) and withdrawal are not acceptable methods of contraception.

# 12. STATISTICAL METHODS

## 12.1. Sample Size and Power

Due to the rare disease setting, the minimal sample size in each dose arm may be determined by feasibility. Depending on possible early termination of 1 or both of the initial 2 arms or the addition of a 3rd dose arm, the study could enroll up to a maximum of 75 subjects.

In order to evaluate the primary objective of safety and tolerability of AG-348 in Arm 1 and Arm 2, up to a maximum of 25 subjects may be randomized onto each arm. The actual number of subjects enrolled into Arms 1 and 2 will depend on the safety reviews and decisions made by the DRT. In addition, up to 25 additional subjects may be enrolled to evaluate an additional dose arm (Arm 3; see Section 7.1).

As for Arms 1 and 2, the actual enrollment in a potential Arm 3 will depend on the safety reviews and decisions made by the DRT. Therefore, up to approximately 75 total subjects may be enrolled in this study across 2 to 3 dose arms.

Table 6 provides the probability within a dose arm of detecting 1 or more AEs with varying sample size and the true underlying AE rates.

**Table 6: Probability of Observing at Least 1 Specific AE Given Different Underlying AE Incidence Rates**

| Sample Size | True Underlying AE Rate | | |
|---|---|---|---|
| | **15%** | **10%** | **5%** |
| 5 | 56% | 41% | 23% |
| 10 | 80% | 65% | 40% |
| 15 | 91% | 79% | 54% |
| 25 | 98% | 93% | 72% |
| 50 | > 99% | 99% | 92% |

Abbreviations: AE = adverse event.

## 12.2. Analysis Sets

The following subject populations (ie, analysis sets) will be evaluated and used for presentation of the data:

- Safety Analysis Set: All subjects who receive at least 1 dose of study treatment. The Safety Analysis Set will be the primary set for the analysis of safety data. Subjects will be classified according to the initial treatment group, defined as the assigned treatment if it is received at least once, or as the first treatment received if assigned treatment is never received.

  Unless otherwise stated, the Safety Analysis Set will be the default analysis set for all data analyses. Additional analysis by actual dose group may be conducted. Details will be provide in the Statistical Analysis Plan (SAP).

- Efficacy Analysis Set: All subjects who are enrolled and received any study drug for at least 3 weeks. The Efficacy Analysis Set will be the primary set for the analysis of preliminary clinical activity data. Subjects will be classified by randomized treatment. Additional analysis by actual dose group may be conducted. Details will be provided in the SAP.

## 12.3. Analysis Periods

Analyses of safety and of indicators of clinical activity will be conducted for the Core Period, and for the Cumulative Period (Core Period and Extension Period), if applicable. Unless specified otherwise, safety analysis will be based on the treatment-emergent period, which is defined as from the first dose to 30 days after the last dose of the corresponding period.

Efficacy analysis will be based on the efficacy window defined as from the first dose to 1 day after the last dose of the corresponding period.

## 12.4. Statistical Analysis

This section presents a summary of the planned statistical analyses of efficacy and safety for this study. Statistical analysis and presentation details will be provided in the SAP for the study, which will be finalized before the database lock after all subjects have completed the Core Period. The results of this analysis will be presented in a clinical study report (CSR). All deviations from the most recent approved SAP before the database lock will be provided in the final CSR.

Additional data collected during the Extension Period after the Core Period database lock will be analyzed for inclusion in a subsequent CSR addendum.

### 12.4.1. General Methods

The primary objective for the Core Period of this Phase 2 study is to evaluate the safety and tolerability of up to 24 weeks of AG-348 in subjects with PK deficiency. Therefore, the analysis of this study will be primarily descriptive in nature; there will be no formal hypothesis testing. Summaries will be produced for disposition, baseline disease characteristics and demographic data.

Categorical variables will be summarized by frequency distributions (number and percentages) and continuous variables will be summarized by descriptive statistics (mean, standard deviation [SD], median, minimum, and maximum).

No imputation will be performed for missing data elements. When summarizing AE data, partial dates will be imputed as described in the SAP. Additional rules addressing the handling of missing data will be detailed in the SAP.

### 12.4.2. Disposition

A summary of the disposition of subjects will be presented, including the number enrolled, the number treated, the number discontinued in the Core Period and the reasons, the number rolled to the Extension period and completed extension, the number discontinued in the Extension period and the reason for discontinuation. Entry criteria and protocol deviations will be listed.

### 12.4.3. Exposure and Safety Analyses

The actual dose and duration in days of AG-348, and the compliance (computed as the ratio of actual dose and the planned dose) will be listed and summarized using descriptive statistics.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and the incidence of AEs occurring in the treatment-emergent period (new or worsening from baseline) will be summarized by primary MedDRA System Organ Class (SOC) and Preferred Term, severity, outcome, action taken with study drug, and relationship to the study drug. Separate summaries will be produced for all TEAEs, treatment-related AEs (those considered by the Investigator as at least possibly study drug-related), SAEs, AESIs, AEs leading to discontinuation, and AEs ≥ Grade 3 severity. Individual subject listings will be provided for deaths, SAEs, and TEAEs leading to treatment modification, interruption, or discontinuation.

Descriptive statistics will be provided for clinical laboratory values (eg, hematology, serum chemistry, coagulation studies, urinalysis) and VS data, presented as both actual values and changes from baseline relative to each on-study evaluation. Shift analyses will be conducted for selected laboratory parameters based on the baseline CTCAE grade to maximum CTCAE grade. Where applicable CTCAE terms do not exist, a grading system based on the upper and/or lower limits of normal will be used to classify the results.

Electrocardiogram analyses will include individual subject listings and summaries of abnormal and clinically significant ECG results. Actual values and changes from baseline in PR, QRS, and heart-rate corrected QT interval (QTc) intervals will be summarized by visit.

Data collected from the menstrual diaries such as the start and stop dates of the menses and the subject reported characteristics of the menses will be presented in a by-subject listing. Additional descriptions of the data may also be performed.

Concomitant medications will be coded using the World Health Organization Drug Dictionary. Concomitant medications prior to and after the start of the study drug will be listed by subject, and summarized by Anatomical Therapeutic Chemical Classification System term and dose arm.

### 12.4.4. Sex Hormone Analysis

Hormone data, including the actual values and their changes from baseline at each visit, will be summarized by sex using descriptive statistics (mean, SD, median, min and max). Spaghetti plots will be provided by sex.

### 12.4.5. Clinical Activity Analyses

Details on analyses to evaluate indicators of potential clinical activity of AG-348 in subjects with PK deficiency will be described in the SAP. These will include changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, hepcidin, ferritin, and transferrin saturation (serum iron/iron-binding capacity).

### 12.4.6. Analysis Specific to the Extension Period

Number and percentage of subjects at individual optimal maintenance dose will be summarized. If necessary, additional analyses related to the dose-taper regimen in the Extension Period, focusing on the long-term treatment effect in the Cumulative Period, may be conducted and will be described in a separate analysis plan after the Core Period database lock, but before the database lock for the Extension Period.

## 12.5. PK/PD Analysis

[redacted]. If such analyses are performed, they will be described in a separate analysis plan and may be reported separately in a standalone report.

### 12.5.1. Pharmacokinetic Analysis

Descriptive statistics will be used to summarize PK parameters for the parent compound AG-348 and the metabolite AGI-8702 for each dose group, and where appropriate, for the entire population. Pharmacokinetic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation Additional PK analyses, if conducted, may be described in a separate analysis plan.

### 12.5.2. Pharmacodynamic Analysis

Descriptive statistics will be used to summarize PD parameters for 2,3-DPG and ATP for each dose group, and where appropriate for the entire population. Pharmacodynamic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation %. Additional PD analyses, if conducted, may be described in a separate analysis plan.

[redacted]

## 12.7. Interim Analysis

No formal statistical interim analysis will be conducted.

Safety data will be reviewed on an ongoing basis by the DRT, who will meet to review safety, PK, PD, and preliminary clinical activity data at regular intervals (approximately every 6 weeks during the Core Period and approximately every 4 to 6 months during the Extension Period once all subjects have completed the Core Period) throughout the duration of the study.

The DRT's decisions to suspend, terminate, or open a potential third dosing arm, or re-assign subjects' dosing in a terminated arm to match the dose and schedule of another arm of the study will be based on the totality of the data including, safety, PK, PD, and preliminary clinical activity (eg, changes in Hb levels). When all the subjects are in the Extension Period, PK/PD data will no longer be reviewed by the DRT.

Additional interim reviews of data may be conducted to support decision making concerning the current clinical study, the Sponsor's development programs in general, or in case of any safety concerns.

# 13. ADMINSTRATIVE REQUIREMENTS

## 13.1. Good Clinical Practice

The study will be conducted in accordance with the ICH for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s). The Investigator will be thoroughly familiar with the appropriate use of the study drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study, and retained according to the appropriate regulations.

## 13.2. Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki (Appendix 5).

The Investigator must obtain IRB approval for the investigation and must submit written documentation of the approval to the Sponsor before he or she can enroll any subject into the study. The IRB will review all appropriate study documentation in order to safeguard the rights, safety, and well-being of the subjects. The study will only be conducted at sites where IRB approval has been obtained. The protocol, Investigator's Brochure, informed consent, advertisements (if applicable), written information given to the subjects, annual progress reports, and any revisions to these documents will be provided to the IRB.

The IRB is to be notified of any amendment to the protocol in accordance with local requirements. Progress reports and notifications of serious unexpected adverse drug reactions are to be provided to the IRB according to local regulations and guidelines.

## 13.3. Subject Information and Informed Consent

The Investigator or trained designee will ensure that the subject is given full and adequate oral and written information about the nature, purpose, possible risk, and benefit of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

After the study has been fully explained, written informed consent will be obtained from the subject prior to study participation.

The subject's signed and dated informed consent must be obtained before conducting any study-related procedures. The Investigator must maintain the original, signed consent form. A copy of the signed form must be given to the subject.

The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

## 13.4. Subject Confidentiality

In order to maintain subject privacy, all source documents, study drug accountability records, study reports and communications will identify the subject by the assigned subject number. The Investigator will grant monitor(s) and auditor(s) from the Sponsor or its designee and regulatory authority(ies) access to the subject's original medical records for verification of data gathered on

the source documents and to audit the data collection process. The subject's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

## 13.5. Protocol Compliance

The Investigator will conduct the study in compliance with the protocol. Modifications to the protocol should not be made without agreement of both the Investigator and the Sponsor. Changes to the protocol will require written IRB approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to subjects. The IRB may provide, if applicable, where regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval/favorable opinion of the IRB. The Sponsor or designee will submit all protocol modifications to the regulatory authority(ies) in accordance with the governing regulations.

When immediate deviation from the protocol is required to eliminate an immediate hazard(s) to subjects, the Investigator will contact the Sponsor's Medical Monitor (or Responsible Medical Officer), if circumstances permit, to discuss the planned course of action. Any departures from the protocol must be fully documented in the source documents/database.

## 13.6. Data Management

A paper or eCRF will be completed for each subject or an electronic data capture (EDC) system will be used. The EDC system (Medidata Rave®) is a software tool designed to ensure quality assurance and facilitate data capture during clinical trials. Through a system regulated workflow that includes barcode scanning and interfaces to medical equipment to avoid manual data entry, study operations performance is controlled and captured in real time. The system is fully Code of Federal Regulations (CFR) 21 Part 11-compliant.

Source documentation supporting the data should indicate participation in the study and should document the dates and details of study procedures, AEs, and subject status. The Investigator, or trained designee should complete and the Investigator should verify the source documents as the information is collected. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.

The Investigator will ensure the accuracy, completeness, and timeliness of the data reported to the Sponsor. Data collection processes and procedures will be reviewed and validated to ensure completeness, accuracy, reliability, and consistency. A complete audit trail will be maintained of all data changes. The Investigator or designee will cooperate with the Sponsor's representative(s) for the periodic review of study documents to ensure the accuracy and completeness of the data capture system at each scheduled monitoring visit.

Electronic consistency checks and manual review will be used to identify any errors or inconsistencies in the data. This information will be provided to the respective study sites by means of electronic or manual queries. The Investigator or designee will prepare and maintain adequate and accurate study documents (medical records, ECGs, AE and concomitant medication reporting, raw data collection forms, et cetera) designed to record all observations and other pertinent data for each subject receiving study treatment.

The Investigator will allow Sponsor representatives, contract designees, authorized regulatory authority inspectors, and the IRB to have direct access to all documents pertaining to the study.

## 13.7. Source Document/Case Report Form Completion

Source documents will be completed for each study subject. It is the Investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the subject's source document. The source document should indicate the subject's participation in the study and should document the dates and details of study procedures, AEs and subject status.

The Investigator, or designated representative, should complete the source document as soon as possible after information is collected, preferably on the same day that a study subject is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.

The Investigator will sign and date each required assessment for all study subjects. The Investigator will retain all completed source documents.

## 13.8. Direct Access to Source Data

The study will be monitored by the Sponsor or its designee. Monitoring will be done by personal visits from a representative of the Sponsor (site monitor) and will include on-site review of the source documents for completeness and clarity, cross-checking with source documents, and clarification of administrative matters will be performed. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.

The site monitor will ensure that the investigation is conducted according to protocol design and regulatory requirements by frequent communications (letter, telephone, e-mail, and fax).

All unused study drug and other study materials are to be returned to the Sponsor or designee after the study has been completed and the database has been locked.

Regulatory authorities, the IRB, and/or the Sponsor's clinical quality assurance group or designee may request access to all source documents, database, and any other applicable study documentation for an on-site audit or inspection. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities.

## 13.9. Record Retention

The Investigator will maintain all study records according to ICH-GCP and applicable regulatory requirement(s). Records will be retained for at least 2 years after the last marketing application approval or 2 years after formal discontinuation of the clinical development of the investigational product or according to applicable regulatory requirement(s). If the Investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility. The Sponsor must be notified in writing if a custodial change occurs.

## 13.10. Liability and Insurance

The Sponsor has subscribed to an insurance policy covering, in its terms and provisions, its legal liability for injuries caused to participating persons and arising out of this research performed

strictly in accordance with the scientific protocol as well as with applicable law and professional standards.

## 13.11. Publication of Study Findings and Use of Information

All information regarding AG-348 supplied by the Sponsor or designee to the Investigator is privileged and confidential information. The Investigator agrees to use this information to accomplish the study and will not use it for other purposes without consent from the Sponsor. It is understood that there is an obligation to provide the Sponsor with complete data obtained during the study. The information obtained from the clinical study will be used towards the development of AG-348 and may be disclosed to regulatory authority(ies), other Investigators, corporate partners, or consultants as required.

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# 15. APPENDICES

# APPENDIX 1: SCHEDULES OF ASSESSMENTS

## Appendix 1.1. Schedule of Assessments: Core Period

| **Timing:**<br><br>**Assessment(s)** | **Pre-treatment** | | **Month 1** | | | | **Months 2 and 3** | | | **Months 4, 5, 6** | | | **Follow-up**[1] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | | **Baseline** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day(s)** | **-42 to -1** | **-40 to -1**[2] | **1** | **8**[3] | **15** | **22**[3] | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window (days)** | **--** | **--** | **--** | **± 2** | **± 2** | **± 2** | **± 7** | **± 7** | **± 7** | **± 7** | **± 7** | **± 7** | **± 7** |
| Written informed consent | X | | | | | | | | | | | | |
| PK enzyme assay[4] (confirmation of PK deficiency) | X | | | | | | | | | | | | |
| PKR genotype (for randomization) | X | | | | | | | | | | | | |
| Demographics | X | | | | | | | | | | | | |
| Medical/surgical history[5] (general and PK deficiency-specific) | X | | | | | | | | | | | | |
| Medication history | X | | | | | | | | | | | | |
| Transfusion history | X | | | | | | | | | | | | |
| Confirmation of vaccinations (splenectomized subjects) | X | | | | | | | | | | | | |
| Physical examination[6]/ Height and weight[6] | X | | X | | X | | | X | X | X | X | X | X |
| ECOG Performance Status | X | | X | | X | | | X | X | X | X | X | X |
| Vital signs[7] (BP, HR, RR, T) | X | | X | X | X | X | X | X | X | X | X | X | X |
| 12-lead ECG[8] | X | | X | X | | X | | | | | | X | X |
| DXA scan | X[9] | | | | | | | | | | | X[10] | |
| Laboratory evaluations[11] | | | | | | | | | | | | | |
| HBsAg, HCV Ab, HIV1 and 2 Ab | X | | | | | | | | | | | | |
| RBC antibody screen | X | | | | | | | | | | | | |
| Hematology (CBC) | X[12] | X[12,13] | X[12] | X | X | X | X | X | X | X | X | X | X |
| Haptoglobin[14] | | | X | | | X | | | X | | | X | X |

| **Timing:**<br><br>**Assessment(s)** | **Pre-treatment** | | **Month 1** | | | | **Months 2 and 3** | | | **Months 4, 5, 6** | | | **Follow-up**[1] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | | **Baseline** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day(s)** | **-42 to -1** | **-40 to -1**[2] | **1** | **8**[3] | **15** | **22**[3] | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window (days)** | **--** | **--** | **--** | **± 2** | **± 2** | **± 2** | **± 7** | **± 7** | **± 7** | **± 7** | **± 7** | **± 7** | **± 7** |
| EPO levels[15] | | | X | | | X | | | X | | | X | X |
| G6PD screen | X | | | | | | | | | | | | |
| Hepcidin | | | X | | | X | | | X | | | X | X |
| Serum chemistry[16] | X | | X | X | X | X | X | X | X | X | X | X | X |
| Iron panel[17] | | | X | | | | | | X | | | X | |
| COHb[18] | | | X | | | X | X | X | X | X | X | X | |
| Coagulation studies[19] | X | | X | | X | | | | X | | | X | X |
| Urinalysis[20] | X | | X | | X | | | | X | | | X | X |
| Serum or urine pregnancy[21] | X | | X | | | | | | | | | | |
| Lipids[22] | | | X | | | | X | | X | | | X | X |
| Hormonal testing[23] | X | X[24] | X | | | | | | X | | | X | X |
| Serum osteocalcin-N-mid and CTX[25] | | | X | | | | | | X | | | X | |
| 25-hydroxy vitamins D2 and D3 | | | X | | | | | | X | | | X | |
| Randomization[26] | X | | | | | | | | | | | | |
| Study drug administration | | | X | X | X | X | X | X | X | X | X | X[27] | |
| Study drug dispensed[28] | | | X | X | X | X | X | X | X | X | X | | |
| PK blood sampling[29] | | | X | | X | X | X | X | X | X | X | X | |
| PD assessments[29] | | | | | | | | | | | | | |
| 2,3-DPG/ATP | | | X | | X | X | X | X | X | X | X | X | |
| [redacted] | | | | | | | | | | | | | |
| Dispense/collect menstrual cycle diary[31] | | | X | | | | X | | X | X | X | X | X |
| Adverse events[32] | | | Continuous | | | | | | | | | | X |
| Transfusion record[33] | X | | X | X | X | X | X | X | X | X | X | X | X |

| **Timing:**<br><br>**Assessment(s)** | **Pre-treatment** | | **Month 1** | | | | **Months 2 and 3** | | | **Months 4, 5, 6** | | | **Follow-up**[1] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | | **Baseline** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day(s)** | **-42 to -1** | **-40 to -1**[2] | **1** | **8**[3] | **15** | **22**[3] | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window (days)** | **--** | **--** | **--** | **± 2** | **± 2** | **± 2** | **± 7** | **± 7** | **± 7** | **± 7** | **± 7** | **± 7** | **± 7** |
| Concomitant medications/procedures | X | | X | X | X | X | X | X | X | X | X | X | X |
| Rollover to Extension Period[3][4] | | | | | | | | | | | | X | |

Abbreviations: 2,3-DPG = 2,3 diphosphoglycerate; Ab = antibody; ATP = adenosine triphosphate; BP = blood pressure; CBC= complete blood count; COHb = carboxyhemoglobin; CTX = C-terminal telopeptide; DPG = diphosphoglycerate; DXA = dual-energy x-ray absorptiometry; ECG = electrocardiogram; ECOG = Eastern Cooperative Oncology Group; EPO = erythropoietin; FSH = follicle-stimulating hormone; G6PD = glucose-6-phosphate-dehydrogenase; HBsAg = hepatitis B surface antigen; HCV Ab = hepatitis C virus antibody; HDL-C = high-density lipoprotein-cholesterol; HIV = human immunodeficiency virus; HR = heart rate; PD = pharmacodynamic; PK = pharmacokinetic; PK deficiency = pyruvate kinase deficiency; PKR = pyruvate kinase isoform R; RBC = red blood cell; RR = resting rate; T = temperature; W = week.

Note: Whenever more than 1 assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (eg, VS, ECG, then blood draw). The timing of these assessments should allow the blood draw to occur at the exact nominal time. The order of procedures may be revised if agreed upon in prior discussion between Sponsor and study site.

1. The Week 28 Follow-up Visit will only be conducted for subjects who do not enter the Extension Period.
2. To be performed at least 2 days after the first Screening Visit.
3. In-clinic visits on Day 8 and Day 22 may be performed by the subject's primary care physician if necessary and must be approved by the Sponsor on a case-by-case basis. In these instances, PK/PD sampling would not be required and dispensing of study medication would not be performed.

   For the Day 8 visit performed by the subject's primary care physician, the primary care medical office will collect a blood sample for hematology using the blood sample collection and shipping supplies from the kit prepared by the designated central laboratory. The kit will be sent to the primary care physician's office. No other testing or procedures will be asked of the primary care physician on Day 8 (VS and serum chemistry will not be required). The 12-lead ECG scheduled for Day 8 will instead be performed at the main study center on Day 15.

   For the Day 22 visit performed by the subject's primary care physician, the primary care medical office will collect a blood sample for hematology using the blood sample collection and shipping supplies from the kit prepared by the designated central laboratory. The kit will be sent to the primary care physician's office. No other testing or procedures will be asked of the primary care physician on Day 22. (VS, serum chemistry, coagulation, haptoglobin, EPO level, carboxyhemoglobin, and PK/PD samples will not be required.) The 12-lead electrocardiogram scheduled for Day 22 will instead be performed at the main study center on Day 43 (Week 6).

   For subjects having their Day 8 and/or Day 22 visits performed by the primary care physician, the Principal Investigator will himself/herself or have a qualified research nurse or other designated site staff member make telephone contact with the subject to inquire about any adverse events. These must be recorded as if the subject appeared in the main study center.

3. (continued) The Principal Investigator will exercise prudent clinical judgment in determining any clinical course of action to take based on any adverse events discovered. These telephone contacts on Day 8 and Day 22 must be explained to the subject in advance and scheduled in advance to maximize the likelihood of successfully making contact.

4. May be performed either by a designated central laboratory or any participating investigative site's local hematology laboratory.

5. Medical history, including all relevant prior medical history, current medical conditions, and hematology profile (CBCs) over prior 6 months, will be obtained at the Screening assessment.

6. A complete physical examination (including neurological examination; genital and rectal examinations will be performed at the discretion of the Investigator) will be obtained at Screening, Baseline, Week 12, and the Follow-up Visit (Week 28), or at Week 24 for subjects rolling over to the Extension Period. The neurological examination must include an assessment of general orientation and mental status including level of alertness (assess as normal or abnormal and specify any abnormality). Limited focused physical examinations will be performed at all other specified visits. Height to be collected at Screening only.

7. Vital signs will include systolic and diastolic blood pressure, heart rate, respiratory rate, and temperature.

8. 12-lead ECGs are to be conducted after 5 minutes of recumbency.

9. If a DXA scan of the hip and spine with T and Z scores has been performed within 3 months preceding the first day of dosing, this may be used to meet this requirement.

10. Week 24 DXA scan may be performed any time between Weeks 24 and 28, and must be performed at the same imaging center on the same DXA machine as the original Screening DXA scan.

11. Laboratory evaluations (hematology, serum chemistry, coagulation studies, and urinalysis) are to be collected in the morning. These should be collected following an overnight fast on Baseline Day 1 Week 6 (Day 43), Week 12 (Day 85), Week 24 (Day 169), and Follow-up Week 28 (Day 197), when the lipid samples are also included.

12. Three Screening/Baseline samples will be collected for complete blood count (CBC). Samples will be collected in the AM on 3 different days; the samples collected on Baseline/ Day 1 may comprise one of these; samples may be taken at the same time as Screening/Baseline hormone assessments (any 2 time points during Screening at least 2 days apart). CBC will include HCT, Hb, red blood cell (RBC) count, absolute reticulocyte count, percent reticulocyte count, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count with automated (or manual, as indicated) differential, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and platelet count.

    In the event that the designated central laboratory for the study is unable to provide a valid result for any specific component of the defined CBC for a specific subject, the site may be asked to have the test performed at their local laboratory. The result of the local CBC will be entered into the study database along with the local normal reference range.

13. The second Screening hematology (CBC) should be drawn in the morning (does not have to be fasting), and may be drawn at the same time the subject returns for the second estradiol and free and total testosterone sample.

14. Haptoglobin will be performed prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

15. Erythropoietin (EPO) levels will be performed prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

16. Alkaline phosphatase, sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide (CO2) or bicarbonate, albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and indirect bilirubin (and estimated creatinine clearance or glomerular filtration rate for Screening only, as appropriate).
17. Iron, total iron-binding capacity (TIBC), transferrin saturation, and ferritin will be performed prior to dosing on Day 1, at the end of Week 12 and at the end of Week 24.
18. To be collected before the AG-348 morning dose is administered.
19. Fibrinogen, activated partial thromboplastin time (aPTT), and international normalized ratio (INR) will be performed at Screening, prior to dosing on Day 1, at the end of Week 2, the end of Week 12, the end of Week 24, and the end of Week 28.
20. Color, appearance, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, and occult blood. Microscopic inspection of sediment should only be performed for cause or to investigate an abnormal dipstick finding per the Investigator's discretion. Urinalysis will be performed at Screening, prior to dosing on Day 1, at the end of Week 2, the end of Week 12, the end of Week 24, and the end of Week 28.
21. Must be repeated at any point throughout the study period if pregnancy is clinically suspected.
22. Total cholesterol, HDL-C, and triglyceride samples will be collected in the morning following an overnight fast.
23. Three Screening/Baseline samples will be collected for estradiol and testosterone (total and free). Samples will be collected in the AM on 3 different days; the samples collected on Baseline/Day 1 may comprise one of these; the Screening samples may be collected at any 2 time points during Screening at least 2 days apart. Serum estrone, estradiol, and free and total testosterone will then follow the schedule indicated on Day 1 and Weeks 12, 24, and 28. FSH will only be performed at Screening for female subjects only for confirmation of postmenopausal status.
24. The second Screening hormone testing will consist of estradiol and testosterone (free and total) only. Samples should be drawn in the AM (does not need to be fasting), and may be drawn at the same time the subject returns for the second CBC sample.
25. Serum osteocalcin-N-mid and CTX will be drawn in the AM each time, approximately between 8-10 AM, and after an overnight fast of 10-12 hours.
26. Randomization will be performed following PKR genotyping and prior to and as close as feasible to dosing on Day 1.
27. Study drug administration is not required on W24/D169 for subjects not continuing into the Extension Period.
28. Study drug will be dispensed on a 28-day schedule, or on an alternate schedule (< 28 days) as needed to accommodate subject visit schedule and dose modifications. The amount of study drug dispensed should be sufficient to provide an adequate reserve supply of AG-348 to ensure uninterrupted dosing in the event of an unexpected delay for the next scheduled study visit (7 extra days of dosing supply is recommended during the Core Period).
29. For the first 10 subjects treated, extensive PK/PD sampling will be conducted on Days 1 and 15 (see for details), followed by limited PK/PD sampling from Week 3 to Week 24 (see Appendix 1.3). Limited PK/PD sampling will be conducted on the remainder of subjects treated (Appendix 1.4). See Section 10.6 and Section 10.7 for details of blood sampling for PK and PD assessments, respectively, and refer to Section 10.9 for sample processing and storage guidelines.

[redacted]

31. Menstruating female subjects will record their menstrual cycles (start, stop, characteristics) monthly. Paper-based menstrual cycle diaries will be dispensed at study visits approximately every month. The previous month's diary will be collected at these visits as well.
32. Randomized subjects will be evaluated for AEs from the time they sign informed consent until they complete all scheduled study follow-up visits or withdraw consent, whichever occurs first.
33. All transfusions must be recorded in the eCRF.
34. Subject must have completed 24 weeks of treatment and tolerated AG-348 (may have had dose modifications). Investigator and Medical Monitor or Responsible Medical Officer must agree with the subject continuing on treatment and subject must sign a separate ICF for the Extension Period.

## Appendix 1.2. Schedule of Assessments: Extension Period

| Visit:<br><br>Assessment(s) | Month 9 | Month 12 | Month 15 | Month 18 | Month 21 | Month 24 | Month 27 | Month 30 | Follow-up |
|---|---|---|---|---|---|---|---|---|---|
| **Approximate Study Day** | **259** | **349** | **439** | **529** | **619** | **709** | **799** | **889** | **919** |
| **Visit Window** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** |
| Physical examination/weight[1] | X | X | X | X | X | X | X | X | X |
| ECOG Performance Status | X | X | X | X | X | X | X | X | X |
| Vital signs (BP, HR, RR, T)[2] | X | X | X | X | X | X | X | X | X |
| 12-lead ECG[3] | | X | | X | | X | | X | X |
| DXA scan | | X | | X | | X | | X | |
| Laboratory evaluations[4] | | | | | | | | | |
| Hematology (CBC)[5] | X | X | X | X | X | X | X | X | X |
| Haptoglobin | | X | | X | | X | | X | X |
| EPO levels[6] | | X | | X | | X | | X | X |
| Hepcidin | | X | | X | | X | | X | |
| Serum chemistry[7] | X | X | X | X | X | X | X | X | X |
| Iron panel[8] | | X | | X | | X | | X | |
| COHb[9] | | X | | X | | X | | X | |
| Coagulation studies[10] | X | X | X | X | X | X | X | X | X |
| Urinalysis[11] | X | X | X | X | X | X | X | X | X |
| Serum or urine pregnancy[12] | | | | | | | | | |
| Lipids[13] | X | X | X | X | X | X | X | X | X |
| Hormonal testing[14] | X | X | X | X | X | X | X | X | X |
| Serum osteocalcin-N-mid and CTX[15] | | | | X | | | | X | |
| Study drug administration | X | X | X | X | X | X | X | X | |
| Study drug dispensed[16] | X | X | X | X | X | X | X | | |
| PK blood sampling[17] | X | X | X | X | X | X | X | X | |
| PD assessments (2,3-DPG/ATP, [redacted])[18] | X | X | X | X | X | X | X | X | |
| Dispense/collect menstrual cycle diary[19] | X | X | X | X | X | X | X | X | X |
| Adverse events[20] | Continuous | | | | | | | | X |
| Transfusion record[21] | X | X | X | X | X | X | X | X | X |
| Concomitant medications/procedures | X | X | X | X | X | X | X | X | X |

Abbreviations: 2,3-DPG = 2,3 diphosphoglycerate; ATP = adenosine triphosphate; BP = blood pressure; CBC= complete blood count; COHb = carboxyhemoglobin; CTX = C-terminal telopeptide; DPG = diphosphoglycerate; DXA = dual-energy x-ray absorptiometry; ECG = electrocardiogram; ECOG = Eastern Cooperative Oncology Group; EPO = erythropoietin; HDL-C = high-density lipoprotein-cholesterol; HIV = human immunodeficiency virus; HR = heart rate; PD = pharmacodynamic; PK = pharmacokinetic; PK deficiency = pyruvate kinase deficiency; PKR = pyruvate kinase isoform R; RBC = red blood cell; RR = resting rate; T = temperature; W = week.

Note: Whenever more than 1 assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (eg, VS, ECG, then blood draw). The timing of these assessments should allow the blood draw to occur at the exact nominal time. The order of procedures may be revised with prior discussion between Sponsor and site.

1. A focused physical examination (including neurological examination; genital and rectal examinations will be performed at the discretion of the Investigator) will be obtained every 3 months; a complete physical examination will be performed at the Month 30 visit. The neurological examination must include an assessment of general orientation and mental status including level of alertness (assess as normal or abnormal and specify any abnormality).
2. Vital signs will include systolic and diastolic blood pressure, heart rate, respiratory rate, and temperature.
3. 12-lead ECGs are to be conducted after 5 minutes of recumbency.
4. Laboratory evaluations (hematology, serum chemistry, coagulation studies, and urinalysis) are to be collected in the morning. These should be collected following an overnight fast.
5. CBC will include HCT, Hb, red blood cell (RBC) count, absolute reticulocyte count, percent reticulocyte count, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count with automated (or manual, as indicated) differential, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and platelet count. In the event that the designated central laboratory for the study is unable to provide a valid result for any specific component of the defined CBC for a specific subject, the site may be asked to have the test performed at their local laboratory. The result of the local CBC will be entered into the study database along with the local normal reference range.
6. Erythropoietin (EPO) levels will be performed prior to dosing at Month 12, Month 18, Month 24, and Month 30.
7. Alkaline phosphatase, sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide (CO2) or bicarbonate, albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and indirect bilirubin.
8. Iron, total iron-binding capacity (TIBC), transferrin saturation, and ferritin will be performed prior to dosing at Month 12, Month 18, Month 24, and Month 30.
9. To be collected before the AG-348 morning dose is administered.
10. Fibrinogen, activated partial thromboplastin time (aPTT), and international normalized ratio (INR) will be performed at each study visit.
11. Color, appearance, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, and occult blood. Microscopic inspection of sediment should only be performed for cause or to investigate an abnormal dipstick finding per the Investigator's discretion. Urinalysis will be performed prior to dosing at each study visit.
12. Must be obtained at any point throughout the study period if pregnancy is clinically suspected. Effective contraception must be continued throughout the Extension Period in all subjects and any pregnancies must be reported (see Section 11.3).
13. Total cholesterol, HDL-C, and triglyceride samples will be collected in the morning following an overnight fast.
14. Serum estrone, estradiol, and free and total testosterone.
15. Serum osteocalcin-N-mid and CTX will be drawn in the AM each time, approximately between 8-10 AM, and after an overnight fast of 10-12 hours.
16. Study drug will be dispensed on a 3-month schedule, or on an alternate schedule (< 3 months) as needed to accommodate subject visit schedule and dose modifications. The amount of study drug dispensed should be sufficient to provide an adequate reserve supply of AG-348 to ensure uninterrupted dosing in the event of an unexpected delay for the next scheduled study visit (14 extra days of dosing supply is recommended during the Extension Period).
17. Predose; PK sampling will only include AG-348 and AGI-8702 concentrations.

18. Predose.

19. Menstruating female subjects will record their menstrual cycles (start, stop, characteristics) monthly. Paper-based menstrual cycle diaries will be dispensed and collected at each study visit.

20. All randomized subjects will be evaluated for AEs from the time they sign informed consent until they complete all scheduled study follow-up visits or withdraw consent, whichever occurs first.

21. All transfusions must be recorded in the eCRF.

## Appendix 1.3. Schedule of Assessments: Extensive PK/PD Sampling during the Core Period

| **Sample Timing/Interval** | **Month 1** | | | | | | | | **Months 2 and 3** | | | **Months 4, 5, 6** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit(s)** | **Baseline/D1 W2/D15** | | | | | | | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** |
| **Study Day(s)** | **1/15** | | | | | | | **22** | **43** | **64** | **85** | **113** | **141** | **169** |
| **Visit Window (days)** | **± 2 (D15)** | | | | | | | **± 2** | **± 7** | **± 7** | **± 7** | **± 7** | **± 7** | **± 7** |
| **Timing:** **Assessment(s)** | Predose[1] | 30 min[2] | 1 hr[2] | 2 hr[2] | 4 hr[3] | 8 hr[3,6] | 12 hr[3,6] | Predose[1] | Predose[1] | Predose[1] | Predose[1] | Predose[1] | Predose[1] | Predose[1] |
| PK blood sample | X | X | X | X | X | X | X[4] | X | X | X | X | X | X | X |
| 2,3 DPG/ATP | X | X | X | X | X | X | X[4] | X | X | X | X | X | X | X |
| [redacted] | | | | | | | | | | | | | | |

Abbreviations: 2,3 DPG = 2,3 diphosphoglycerate; ATP = adenosine triphosphate; D = day; DPG = diphosphoglycerate; hr = hours; min = minutes; PD = pharmacodynamic; PK = pharmacokinetic; PKM2 = pyruvate kinase isoform M2; PKR = pyruvate kinase isoform R; W = week.

1. The acceptable time window will be within 60 minutes prior to study treatment dose administration for the predose PK/PD sample. Study drug administration is not required on W24/ D169 for subjects not continuing into the Extension Period.
2. The acceptable time window will be within ± 5 minutes of the scheduled collection time for the 30-minute, 1-hour, and 2-hour PK/PD samples.
3. The acceptable time window will be within ± 30 minutes of the scheduled collection time for the 4, 8, and 12-hour PK/PD samples.
4. To be collected on Day 1 only.
5. [redacted]
6. If the 12-hour time point sample cannot be collected at site on Day 1, an 8-hour time point sample may be collected instead.
7. [redacted]

## Appendix 1.4. Schedule of Assessments: Limited PK/PD Sampling during the Core Period

| Sample Timing/ Interval | Month 1 | | | Months 2 and 3 | | | Months 4, 5, 6 | | |
|---|---|---|---|---|---|---|---|---|---|
| **Visit(s)** | **Baseline/ D1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** |
| **Study Day** | **1** | **15** | **22** | **43** | **64** | **85** | **113** | **141** | **169** |
| **Visit Window (days)** | **--** | **± 2** | **± 2** | **± 2** | **± 7** | **± 7** | **± 7** | **± 7** | **± 7** |
| **Timing:** | Predose[1] | Predose[1] | Predose[1] | Predose[1] | Predose[1] | Predose[1] | Predose[1] | Predose[1] | Predose[1] |
| **Assessment(s)** | | | | | | | | | |
| PK blood sample | X | X | X | X | X | X | X | X | X |
| 2,3 DPG/ATP | X | X | X | X | X | X | X | X | X |
| [redacted] | | | | | | | | | |

Abbreviations: 2,3 DPG = 2,3 diphosphoglycerate; ATP = adenosine triphosphate; D = day; DPG = diphosphoglycerate; hr = hours; min = minutes; PD = pharmacodynamic; PK = pharmacokinetic; PKM2 = pyruvate kinase isoform M2; PKR = pyruvate kinase isoform R; W = week.

1. The predose blood sample for plasma PK/PD analysis should be collected within 60 minutes prior to study treatment dose administration.

[redacted]

# APPENDIX 2: EASTERN COOPERATIVE ONCOLOGY GROUP PERFORMANCE STATUS SCORING

| Grade | Symptomatology |
|---|---|
| 0 | Fully active, able to carry on all predisease performance without restriction. |
| 1 | Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, eg, light house work, office work. |
| 2 | Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours. |
| 3 | Capable of only limited self-care, confined to bed or chair more than 50% of waking hours. |
| 4 | Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair. |
| 5 | Dead. |

Source: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and Response Criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-655.

# APPENDIX 3: NATIONAL CANCER INSTITUTE COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS

The NCI CTCAE, Version 4.03, can be accessed using the following link:

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

# APPENDIX 4: CONCOMITANT THERAPY TO BE AVOIDED (PROHIBITED) OR REQUIRING CAREFUL MONITORING (USE WITH CAUTION)

## Appendix 4.1. Strong and Moderate CYP3A4 Inhibitors

| Strong CYP3A4 Inhibitors: Prohibited | Moderate CYP3A4 Inhibitors: Substitute or Use with Caution |
|---|---|
| Boceprevir | Amprenavir |
| Clarithromycin | Aprepitant |
| Conivaptan | Atazanavir |
| Grapefruit and grapefruit juice[2] | Ciprofloxacin |
| Indinavir | Darunavir |
| Itraconazole | Diltiazem[1] |
| Ketoconazole | Erythromycin[1] |
| Ritonavir/Lopinavir | Fluconazole |
| Mibefradil (withdrawn from US market) | Fosamprenavir |
| Nefazodone | Verapamil[1] |
| Nelfinavir | |
| Posaconazole, | |
| Saquinavir | |
| Suboxone | |
| Telaprevir, | |
| Telithromycin | |
| Voriconazole | |

Note: Strong Inhibitor = > 5-fold increase in area under the curve (AUC); Moderate Inhibitor = > 2-fold, < 5-fold increase in AUC.

1. Erythromycin, verapamil, and diltiazem are contraindicated because they are strong P-gp inhibitors (see Appendix 4.3).
2. Although grapefruit is classified as a moderate CYP3A4 inhibitor, it is prohibited, as is grapefruit juice.

## Appendix 4.2. Strong CYP3A4 Inducers (Prohibited)

| |
|---|
| Carbamazepine |
| Oxcarbazepine |
| Phenobarbital |
| Phenytoin |
| Rifabutin |
| Rifampin |
| St. John's Wort |

Note: Corticosteroids may induce CYP3A4. Although the use of corticosteroids is not prohibited, their use should be minimized as much as is medically feasible.

## Appendix 4.3. Strong P-glycoprotein Inhibitors (Prohibited)

| | |
|---|---|
| Amiodarone | Felodipine |
| Azithromycin | Itraconazole |
| Captopril | Ketoconazole |
| Carvedilol | Lopinavir |
| Clarithromycin | Ritonavir |
| Conivaptan | Quercetin |
| Cyclosporine | Quinidine |
| Diltiazem | Ranolazine |
| Dronedarone | Ticagrelor |
| Erythromycin | Verapamil |

## Appendix 4.4. Sensitive CYP3A4 Substrates (Substitute or Use with Caution)

| **Benzodiazepines:** | **Antihistamines:** | **Miscellaneous:** | | |
|---|---|---|---|---|
| | Chlorpheniramine | Alfentanil | Finasteride | Salmeterol |
| | | Aprepitant | Gleevec | Sildenafil |
| | **Calcium Channel Blockers:** | Aripiprazole | Haloperidol | Sirolimus |
| Alprazolam | Amlodipine | Boceprevir | Irinotecan | Sorafenib |
| Diazepam→3OH | Lercanidipine | Buspirone | LAAM | Sunitinib |
| Midazolam | Nifedipine | Cafergot | Lidocaine | Tamoxifen |
| Triazolam | Nisoldipine | Caffeine→TMU | Methadone | Taxol |
| | Nitrendipine | Cilostazol | Nateglinide | Telaprevir |
| **Immune Modulators:** | | Cocaine | Nevirapine | Terfenadine |
| Tacrolimus (FK506) | **HMG CoA Reductase Inhibitors**: | Codeine-N-demethylation | Ondansetron | Torisel |
| | Atorvastatin | Dapsone | Pimozide | Trazodone |
| **Steroid 6beta-OH:** | Cerivastatin | Dextromethorphan | Propranolol | Vemurafenib |
| Estradiol | Lovastatin | Docetaxel | Quetiapine | Vincristine |
| Hydrocortisone (and other glucocorticoids) | Simvastatin | Domperidone | Quinine | Zaleplon |
| Progesterone | | Eplerenone | Risperidone | Ziprasidone |
| Testosterone | | Fentanyl | Romidepsin | Zolpidem |

## Appendix 4.5. Sensitive CYP2B6 Substrates* (Substitute or Use with Caution)

| Bupropion | Efavirenz |
|---|---|

* The areas under the curve of these substrates were not increased by 5-fold or more with a CYP2B6 inhibitor, but they represent the most sensitive substrates studied with available inhibitors evaluated to date.

## Appendix 4.6. Proton-Pump Inhibitors and H2-Receptor Antagonists (Substitute or Use with Caution)

| **Proton-pump inhibitors:** | |
|---|---|
| Dexlansoprazole | Omeprazole |
| Esomeprazole | Rabeprazole |
| Lansoprazole | Pantoprazole |
| **H2-receptor antagonists:** | |
| Cimetidine | Nizatidine |
| Famotidine | Ranitidine |

# APPENDIX 5: DECLARATION OF HELSINKI

## WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI:

Recommendations Guiding Medical Doctors in Biomedical Research Involving Human Subjects

Adopted by the 18th World Medical Association (WMA) General Assembly, Helsinki, Finland, June 1964 and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975, 35th WMA General Assembly, Venice, Italy, October 1983, and the 41st WMA General Assembly, Hong Kong, September 1989, the 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996; 52nd WMA General Assembly, Edinburgh, Scotland, October 2000; 53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added); 55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added); and 59th WMA General Assembly, Seoul, October 2008.

### A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.
   The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of subjects, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my subject will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the subject's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
8. In medical practice and in medical research, most interventions involve risks and burdens.

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.

10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

**B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH**

1. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

2. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

3. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.

4. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, Sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for poststudy access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.

5. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the Sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any SAEs. No change to the protocol may be made without consideration and approval by the committee.

6. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research in subjects with a particular diagnosis or healthy adult subjects requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for

the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

7. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
8. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
9. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
10. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
11. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
12. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
13. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
14. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the nonwritten consent must be formally documented and witnessed.
15. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

16. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.

17. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.

18. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

19. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious subjects, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.

20. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

## C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

1. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the subjects who serve as research subjects.

2. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:

    - The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists.
    - Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the subjects who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

3. At the conclusion of the study, subjects entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

4. The physician must fully inform the subject which aspects of the care are related to the research. The refusal of a subject to participate in a study or the subject's decision to withdraw from the study must never interfere with the subject-physician relationship.

5. In the treatment of a subject, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the subject or a legally authorized representative, may use an unproven intervention if, in the physician's judgment, it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

# Clinical Study Protocol AG348-C-003
# EudraCT No. 2015-000484-13
# A Phase 2, Open Label, Randomized, Dose Ranging, Safety, Efficacy, Pharmacokinetic and Pharmacodynamic Study of AG-348 in Adult Patients with Pyruvate Kinase Deficiency

| | |
|---|---|
| Study Sponsor: | Agios Pharmaceuticals, Inc.<br>88 Sidney Street<br>Cambridge, MA 02139-4169<br>Phone: 617-649-8600<br>Fax: 617-649-8618 |
| Responsible Medical Officer: | [redacted], MD, PhD<br>[redacted]<br>Agios Pharmaceuticals, Inc.<br>Phone: [redacted]<br>Email: [redacted] |
| Study Medical Monitor | [redacted], MD<br>[redacted]<br>On behalf of Agios Pharmaceuticals, Inc.<br>Mobile Phone: [redacted]<br>Office Phone: [redacted]<br>Email: [redacted] |
| Document Version (Date):<br>Revised | Version 1.0 (05 January 2015)<br>Amendment 1, Protocol Version 2.0 (02 February 2015) Final<br>Amendment 2, Protocol Version 3.0 (05 August 2015) Final<br>Amendment 3, Protocol Version 4.0 (10 November 2015) Final<br>Amendment 4, Protocol Version 5.0 (30 March 2016) Final |

This study will be conducted according to the protocol and in compliance with Good Clinical Practice, the ethical principles stated in the Declaration of Helsinki, and other applicable regulatory requirements.

**CONFIDENTIALITY NOTE:**

The information contained in this document is confidential and proprietary to Agios Pharmaceuticals, Inc. Any distribution, copying, or disclosure is strictly prohibited unless such disclosure is required by federal regulations or state law. Persons to whom the information is disclosed must know that it is confidential and that it may not be further disclosed by them.

# INVESTIGATOR'S AGREEMENT

I understand that all documentation provided to me by Agios or its designated representative(s) concerning this study that has not been published previously will be kept in strict confidence. This documentation includes the study protocol, Investigator's Brochure, case report forms, and other scientific data.

This study will not commence without the prior written approval of a properly constituted Institutional Review Board (IRB). No changes will be made to the study protocol without the prior written approval of Agios and the IRB, except where necessary to eliminate an immediate hazard to the patient.

I have read, understood, and agree to conduct this study as outlined in the protocol and in accordance with the guidelines and all applicable government regulations.

| Investigator Name (printed) | Investigator Signature | Date |
|---|---|---|

Investigational site or name of institution and location (printed)

# 2. SYNOPSIS

**Name of Sponsor/Company:**
Agios Pharmaceuticals, Inc.

**Name of Investigational Product:**
AG-348

**Name of Active Ingredient:**
AG-348 sulfate hydrate

**Title of Study:**
A Phase 2, Open Label, Randomized, Dose Ranging, Safety, Efficacy, Pharmacokinetic and Pharmacodynamic Study of AG-348 in Adult Patients with Pyruvate Kinase Deficiency

**Study Center(s):**
This study will be conducted at multiple study centers.

**Phase of Development:** 2

***Objectives:***

**Core Period**

**Primary:**

- Evaluate the safety and tolerability of up to 24 weeks of AG-348 administration in patients with pyruvate kinase deficiency (PK deficiency).

**Secondary:**

- Evaluate the pharmacokinetics (PK) of AG-348 and the metabolite AGI-8702.
- Evaluate the pharmacodynamic (PD) response of adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG) after administration of AG-348.
- Evaluate indicators of clinical activity of AG-348 in patients with PK deficiency, including changes in hemoglobin (Hb), hematocrit (HCT), reticulocyte count, haptoglobin (Hp), carboxyhemoglobin (COHb), end tidal carbon monoxide (ETCO), lactate dehydrogenase (LDH), total and indirect bilirubin, erythropoietin (EPO), hepcidin, ferritin, and transferrin saturation (serum iron/iron binding capacity).

[redacted]

**Extension Period**

**Primary:**

- Evaluate the safety and tolerability of up to 30 months of AG-348 administration in patients with PK deficiency.

**Secondary:**

- Evaluate the PK of AG-348 and the metabolite AGI-8702.
- Evaluate the PD response of ATP and 2,3-DPG after administration of AG-348.

**Name of Sponsor/Company:**
Agios Pharmaceuticals, Inc.

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- Evaluate indicators of clinical activity of AG-348 in patients with PK deficiency, including changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, hepcidin, ferritin, and transferrin saturation (serum iron/iron binding capacity).

[redacted]

**Methodology:**

Study AG348-C-003 is a Phase 2, open label, two arm, multicenter, randomized, dose-ranging study in adult patients with PK deficiency; the study will be divided in to a Core Period and an Extension Period. During the Core Period, patients will receive multiple doses of AG-348 for up to 24 weeks; patients who are eligible can enter the Extension Period to receive AG-348 for up to 2 years following the end of the Core Period. Patients with PK deficiency confirmed by red blood cell (RBC) PK enzymatic assay performed at Screening will be eligible to participate in this study. At Week 25, patients who safely tolerate AG-348 and demonstrate clinical activity of AG-348 may be eligible to immediately roll over to the Extension Period for continued treatment. Patients who complete treatment at the end of the Core Period (24 weeks) will undergo follow-up assessment 4 weeks after the last dose of study drug. If a patient discontinues at any other time (including early discontinuation or discontinuation during the Core or Extension Period), the follow-up assessments will be conducted 4 weeks after discontinuation. Patients with toxicity suspected to be related to study drug will continue follow-up until the adverse event (AE) resolves, is declared chronic by the Investigator, or the patient is lost to follow-up.

For the Core Period, up to 25 patients will be initially randomized on an open-label 1:1 basis to each of two twice-daily (BID) doses of AG-348 (up to 50 patients total; see Figure 1, Study Schema). The dose of Arm 1 is 300 mg of AG-348 administered orally (PO) every 12 hours (q12h, BID). The dose of Arm 2 is 50 mg of AG-348 administered PO q12h (BID). Randomization will be stratified by PKR mutation in order to maintain balance as much as possible across the dose arms for the specific mutations expected to be most frequently enrolled. The PKR mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H) and all other mutations ("other"). Mutation status is defined by the presence of at least one of the indicated mutations; patients with more than one stratified mutation will be assigned based on Sponsor's discretion.

The doses for each arm of the Core Period have been selected from the AG348-C-001 single ascending dose (SAD) and AG348-C-002 multiple ascending dose (MAD) studies in healthy adult volunteers to represent the range of doses/exposures that were safely tolerated and resulted in maximal or near-maximal PD effects on 2,3-DPG and ATP.

Because PK deficiency is a rare disease with a limited eligible patient population and because the underlying pathophysiology and clinical phenotype of affected patients is heterogeneous due to the wide variety of mutations in PKR that cause the disease, it is important to focus closely on dose findings in this first-in-patient study. Therefore, in addition to initiating this study with 2 different doses of AG-348 administered q12h, a Data Review Team (DRT) will be established to review study data on a regular basis and adapt the study design, dose and schedule of AG-348.

**Name of Sponsor/Company:**
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The DRT will monitor safety on an on-going basis and meet at regular intervals of approximately every 6 weeks, or *ad hoc*, as necessary, for as long as any patients are still in the Core Period to review AEs, vital signs (VS), clinical laboratory assessments (hematology, clinical chemistry, coagulation, and urinalysis), and electrocardiograms (ECGs). The DRT will also review available PK/PD data and indicators of clinical activity (e.g., changes from baseline in Hb). These DRT meetings will also include data review for all patients that may be under treatment in the Extension Period. If there are no patients still being treated in the Core Period, and the only patients being treated are those in the Extension Period, then the frequency of the DRT meetings will reduce to approximately every 3 months in order to match the frequency of patient visits (and new data collection) in the Extension Period. The DRT will be comprised of the study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer.

Beginning 6 weeks after the first patient is dosed in the Core Period or *ad hoc* as necessary, and proceeding according to the schedule indicated above (approximately every 6 weeks during the Core Period, and approximately every 3 months during the Extension Period once all patients have completed the Core Period), the DRT will review cumulative safety data, available PK/PD data, and clinical activity data. Based on the DRT's recurring reviews, the DRT may exercise one or more of the following options during the Core Period:

- Continue treatment and enrollment in existing arms without change.
- Add 1 new dose arm (Arm 3) to enroll up to 25 patients at a dose to be determined; the dose for Arm 3 may be lower or higher than Arm 1 and Arm 2 doses, but will not exceed 360 mg q12h; and the dose regimen may be less frequent than q12h.
- Terminate or suspend enrollment to allow further review of clinical data in Arm 1 and/or Arm 2 (and/or potential Arm 3). Enrollment in an arm could be terminated or suspended to allow further review, for example, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity.
- Re-assign patients' doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the patients in the terminated arm will remain in their original arm, i.e., they will not count towards the enrollment quota of the arm whose dose and schedule is being adopted.
- Implement specific genotype restrictions to enrollment in one or more arms to ensure representation of patients with genotypes of greatest clinical relevance.

The DRT may exercise one or more of the following options during the Extension Period:

- Continue treatment without change;
- Re-assign patients' doses and schedule to an existing dose and schedule that has been determined to be safer, and/or have a better PD response, or produce signs of clinical activity;
- Terminate or suspend treatment to allow further review of clinical data (eg, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity).

The data that the DRT will review to make these decisions is expected to include, but are not necessarily limited to, the following:

| |
|---|
| **Name of Sponsor/Company:**<br>Agios Pharmaceuticals, Inc. |
| **Name of Investigational Product:**<br>AG-348 |
| **Name of Active Ingredient:**<br>AG-348 sulfate hydrate |

- *Safety Observations:* all AEs; VS, clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and ECG;
- *PK and PD Observations:* including changes in 2,3-DPG and ATP;
- *Indicators of Clinical Activity:* including changes in Hb, HCT, reticulocyte count, Hp, COHb, ETCO (Core Period only), LDH, EPO, hepcidin, total and indirect bilirubin, ferritin, and transferrin saturation.

If a third dose arm is implemented, the dose of AG-348 selected will not exceed 360 mg BID, as this was the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy volunteers. The PK/PD sampling schedule in a potential third dosing arm will be determined by Sponsor's discretion and may follow either the extensive or limited PK/PD sampling schedules as specified.

The DRT will monitor the data in an ongoing manner as described and may make a decision to terminate enrollment in an arm if unacceptable AEs are observed, PD activity is limited, and/or markers of clinical activity indicate no effect. Unacceptable AEs are defined as ≥ Grade 3 AEs (using National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAEv4.03]) that are assessed as at least possibly related to AG-348, with the exception of Grade 3 headache, hot flash/flushing, nausea, vomiting, and/or diarrhea that is transient in duration (< 24 hours) or able to be medically managed to ≤ Grade 2 within 24 hours. The DRT may also exercise discretion to terminate enrollment in an arm if it observes a frequency of Grade 2 AEs that would make it difficult to support long-term dosing.

Patients in the Extension Period will continue on the dose they were randomized to in the Core Period, unless the DRT had reason to establish a different dose/schedule during the course of the Core Period (the DRT will not propose a dose higher than 360 mg q12h), or unless the treating Investigator exercises the option for intra-patient dose escalation (see Section 9.7.2).

Due to the potential for AG-348-mediated aromatase inhibition, dual-energy x-ray absorptiometry (DXA) scan (hip and spine) will be performed at Screening (if patient has not had prior DXA scan within 3 months of Day 1) to obtain T and Z scores. These data are intended to serve as a baseline measure of bone mineral density for all enrolling patients, and are deemed of particular importance for those who enter the longer term Extension Period after completing 24 weeks of treatment (Core Period). All patients will have a second DXA scan in the interval between Weeks 24 and 28 for the Core Period. Patients in the Extension Period will have additional DXA scans at Months 18 and 30.

As the number of enrolling arms changes in the study (for example, from 2 to 3), the randomization scheme will adjust to enable balanced randomization into each actively accruing arm. Randomization and stratification will cease in the event that only a single arm is left enrolling.

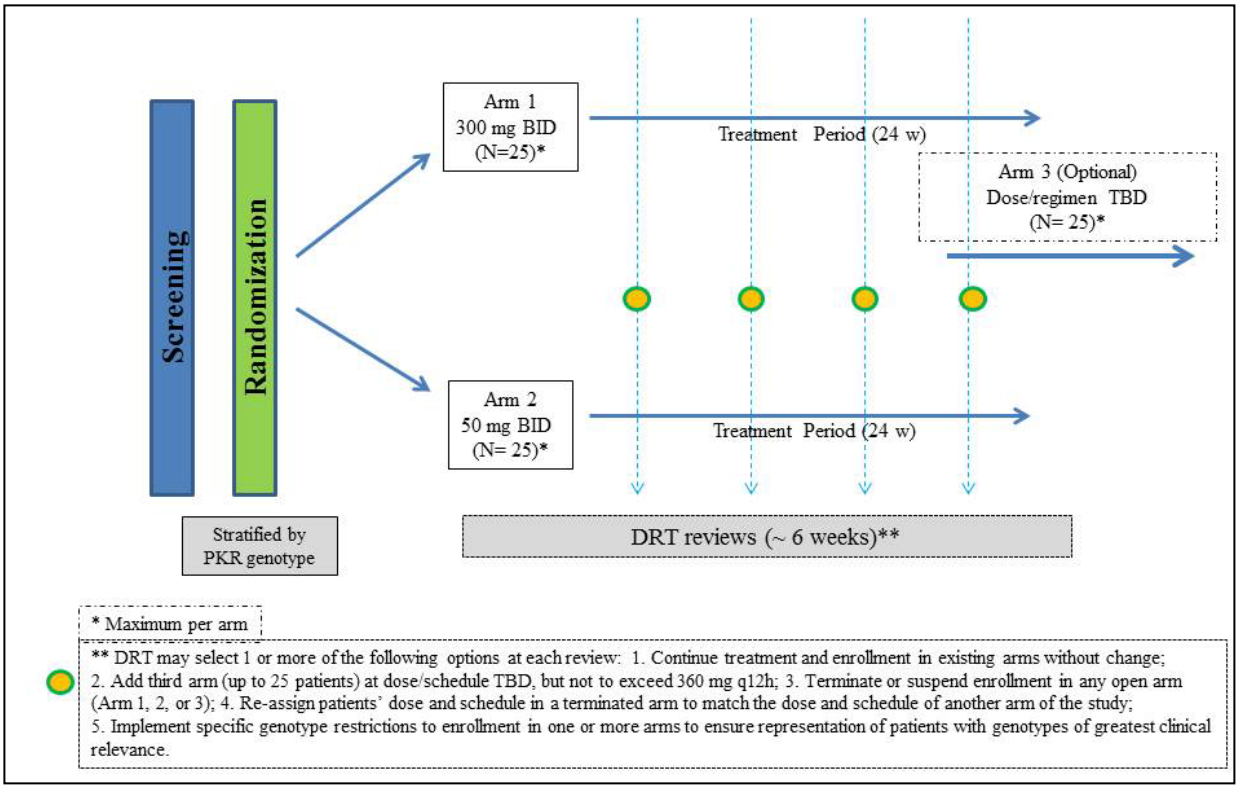
Depending on possible early termination of 1 or both of the initial 2 arms, or the addition of a third arm, the study could enroll up to a maximum of 75 patients.

**Name of Sponsor/Company:**
Agios Pharmaceuticals, Inc.

**Name of Investigational Product:**
AG-348

**Name of Active Ingredient:**
AG-348 sulfate hydrate

**Figure 1: Study Schema: Core Period**




Abbreviations: BID (q12h) = twice-daily (every 12 hours); DRT = data review team; PKR = pyruvate kinase red blood cell isoform; TBD= to be determined; w = week(s).

***Visit Schedule***

Screening assessments will occur within 42 days prior to the first dose of study treatment. During the Core Treatment Period, patients will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12), and monthly through Week 24 (Weeks 16, 20, and 24). Patients who safely tolerate AG-348 of AG-348 through Week 24 (Core Period) may be eligible to immediately enter the Extension Period for continued treatment for up to 2 years after completion of the Core Period upon agreement of the treating Investigator and the Medical Monitor or Responsible Medical Officer. Study visits for safety and clinical activity assessments will occur approximately every 3 months during the Extension Period. All patients will undergo a follow-up assessment 4 weeks after the last dose of AG-348, regardless of whether this was due to early discontinuation, the last dose in the Core Period for a patient who chooses not to continue in the Extension Period, or the last dose of the Extension Period.

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**Dose Modifications for Safety and/or Increase in Hb Level**
The Investigator will monitor all patients for safety and tolerability. Modification of an individual patient's dose of AG-348 will be based on AEs and/or observed changes in Hb level as described in Section 9.7.1 and Section 9.7.2. The same criteria for dose modifications will apply in the Extension Period as in the Core Period.

**Number of patients (planned):** Up to approximately 75 patients.

**Diagnosis and main criteria for inclusion:**

**Inclusion criteria:**

For entry into the Core Period, patients must meet all of the following criteria during the Screening or other specified period:

1. Signed written informed consent obtained prior to performing any study procedure, including screening procedures.
2. Male or female, aged 18 years and older.
3. Known medical history of PK deficiency.
4. All patients must have documented clinical laboratory confirmation of PK deficiency by RBC pyruvate kinase enzymatic assay performed at Screening either by a designated central laboratory or by any participating investigative site's local hematology laboratory. Patients with prior documentation of PK deficiency by RBC enzymatic assay will have a reconfirmation of this result during Screening as a condition of enrollment.
    i. In the event that a patient's screening pyruvate kinase enzymatic assay is negative (i.e., shows normal pyruvate kinase activity), the patient will be eligible for enrollment if the genotyping shows a mutant genotype that has been previously documented in the literature to be associated with PK deficiency. If the genotyping shows a previously undescribed mutation in the PKR gene, then the eligibility for enrollment will be determined on a case-by-case basis by the Coordinating Investigator and Medical Monitor in discussion with the Investigator. If no mutation is defined, then the patient will not be eligible.
5. ALL patients must have a blood sample for genotypic characterization of the mutant PKR gene performed by the designated central laboratory at Screening. The designated central laboratory-determined genotype will generally serve as the basis for genotyping for enrollment. However, patients whose genotype has already been determined by another laboratory may be enrolled on the basis of that report, with the approval of the Medical Monitor, in case of unexpected delay in return of the designated central laboratory result during the Screening Period. Enrollment on the basis of a result from a laboratory other than the designated central genotyping laboratory does not relieve the inclusion requirement that ALL patients must have a sample sent to the

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designated central genotyping laboratory.

6. All patients must have genotypic characterization of the UGT1A1 gene performed by a designated central laboratory to document whether they may have underlying Gilbert's Disease. Patients with Gilbert's Disease are eligible to enroll.
7. Males must have Hb ≤ 12.0 g/dL; females must have Hb ≤ 11.0 g/dL.
8. All patients must be considered transfusion independent as defined by: no greater than 3 units of RBCs transfused in the 12-month period up to the first day of study dosing and no transfusions within 4 months of first day of study dosing. Patients who have received more transfusion support than described above will be evaluated for eligibility on a case-by-case basis by the Medical Monitor.
9. Eligible patients may still have their spleens in place, or may have undergone prior splenectomy. For splenectomized patients:
    i. Must have undergone their procedure at least 6 months prior to Screening.
    ii. Must be current in their vaccinations for Pneumococcal Conjugate (PCV13), Pneumococcal Polysaccharide (PPSV23), Quadrivalent Meningococcal vaccine, and *Haemophilus influenzae* type b as recommended by Centers for Disease Control and Prevention's (CDC) Advisory Committee on Immunization Practices (ACIP) or immunization advisory groups in Canada and the European Union (for patients enrolled in Canada and the EU). [http://www.cdc.gov/vaccines/schedules/downloads/adult/adult-schedule.pdf] [Any missing vaccinations may be administered starting with the Screening Period and during the trial following the initiation of AG-348 dosing as necessary according to recommended vaccination guidance.]
10. Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 2. (Appendix 15.2)
11. Patients must be taking at least 1 mg of folic acid daily for at least 21 days prior to first dose and continue daily during study participation.
12. Adequate organ function, defined as:
    i. Serum aspartate aminotransferase (AST) ≤ 2.5 × upper limit of normal (ULN; unless the increased AST is assessed by the Investigator as due to hemolysis and/or hepatic iron deposition); and alanine aminotransferase (ALT) ≤ 2.5 × ULN (unless the increased ALT is assessed by the Investigator as due to hepatic iron deposition).
    ii. Normal or elevated levels of serum bilirubin. In patients with serum bilirubin > ULN, the elevation must be attributed to hemolysis with or without Gilbert's syndrome and must not be choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease.
    iii. Serum creatinine ≤ 1.25 × ULN. If serum creatinine > 1.25 × ULN, then 24-hour measured or calculated (Cockcroft-Gault) glomerular filtration rate (GFR) ≥ 60 mL/min.

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iv. Absolute neutrophil count (ANC) $\geq 1.0 \times 10^9$/L.
v. Platelet count $\geq 100 \times 10^9$/L.
vi. Activated partial thromboplastin time (aPTT) and international normalized ratio (INR) $\leq 1.25 \times$ ULN, unless the patient is receiving therapeutic anticoagulants.

13. Women of childbearing potential (WOCBP) must agree to abstain from sexual intercourse or to use an acceptable/effective method of contraception (i.e., condom plus spermicide, condom plus oral contraceptive, condom plus intrauterine device [IUD], condom plus diaphragm with spermicide) from as soon as feasible during the Screening period until 30 days following the last dose of AG-348. Abstinence is an acceptable method only when this is in line with the normal life style of the patient, meaning that the patient plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence, e.g., calendar, sympathothermal and post-ovulation methods, and withdrawal are not acceptable methods of contraception.

    i. WOCBP include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, and/or bilateral oophorectomy) or is not post-menopausal. Post-menopausal is defined as:
    ii. Amenorrhea $\geq$ 12 consecutive months without another cause, and a documented serum follicle stimulating hormone (FSH) level > 35 mIU/mL;
    iii. Amenorrhea $\geq$ 12 consecutive months in women $\geq$ 62 years old (FSH testing is not required).

14. WOCBP must have a negative serum or urine pregnancy test within 72 hours before start of AG-348 dosing.

15. Women must not be breastfeeding.

16. Male patients, with the exception of those who have undergone vasectomy at least 6 months prior to Screening, must agree to abstain from sexual intercourse or, if sexually active, to use a condom with spermicide as contraception (regardless of their female partner's childbearing potential or their partner's use of their own contraception) from Day 1 of dosing until 30 days following the last dose of AG-348. Abstinence is an acceptable method only when this is in line with the normal life style of the patient, meaning that the patient plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence, e.g., selective timing of intercourse based on partner's calendar, sympathothermal and post-ovulation methods, and withdrawal are not acceptable methods of contraception.

For entry into the Extension Period, patients must meet the following criteria:

17. Signed written informed consent obtained prior to performing any study procedure during the Extension Period.

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18. Patient must have completed 24 weeks of treatment during the Core Period and tolerated AG-348 (defined as having completed 24 weeks with or without permitted dose modifications)
19. The patient's treating Investigator agrees that there is a potential for clinical benefit to continued treatment and recommends participation in the Extension Period
20. The Sponsor's designated Medical Monitor or Responsible Medical Officer approves the patient's participation in the Extension Period
21. As applicable, the patient must agree to continue to follow the same sexual abstinence/contraception rules as stated in Inclusion Criteria 13 and 16.

**Exclusion criteria:**

Patients who meet any of the following criteria at Screening or prior to dosing on Day 1 will not be enrolled in the Core Period:

1. Hemoglobin level > 12.0 g/dL if male; Hb > 11.0 g/dL if female.
2. Additional diagnosis of any other congenital or acquired blood disorder, including glucose-6-phosphate-dehydrogenase (G6PD) deficiency, or any other hemolytic anemia process except for mild allo-immunization as a consequence of transfusion therapy.
3. Iron overload (hemosiderosis or concurrent hemochromatosis) sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac, hepatic, or pancreatic insufficiency.
4. Prior bone marrow or stem cell transplant.
5. Clinically symptomatic cholelithiasis or cholecystitis. (Prior cholecystectomy is not exclusionary. Patients with symptomatic cholelithiasis or cholecystitis may be rescreened once the disorder has been treated and clinical symptoms have resolved.)
6. Currently enrolled in another therapeutic clinical trial involving on-going therapy with any investigational or marketed product or placebo. Concurrent participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480) is permitted.
7. Exposure to any investigational drug, device, or procedure within 28 days prior to Screening or during trial participation.
8. Concurrent medical condition that could compromise participation in the study such as:
    a. Poorly controlled hypertension (defined as systolic blood pressure (BP) > 150 mm Hg or diastolic BP > 90 mm Hg) refractory to medical management.
    b. History of recent (within < 6 months from Screening date) congestive heart failure; myocardial infarction or unstable angina pectoris; or hemorrhagic, embolic, or

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thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.

c. Currently active infection requiring the use of parenteral anti-microbial agents or that is greater than Grade 3 (CTCAEv4.03) within 6 months of first dose.

d. A pattern or frequency of post-splenectomy sepsis that in the assessment of the Investigator could reasonably be expected to interfere with the ability of the patient to complete the 24 week Core Period study participation.

e. Positive test for hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV) antibody with signs of active Hepatitis B or C virus infection.

f. Positive test for human immunodeficiency virus (HIV) 1 or 2 antibody.

g. Diabetes mellitus judged to be in poor control by the Investigator or requiring > 3 anti-diabetic agents counting insulin (all insulins are considered one agent); use of insulin per se is not exclusionary.

h. History of any primary malignancy with the exception of: curatively treated non-melanomatous skin cancer; curatively treated cervical or breast carcinoma in situ; or other primary tumor treated with curative intent and no known active disease present and no treatment administered during the last 3 years.

9. Undergone major surgery within 6 months of first dose.

10. Current or recent history of psychiatric disorder that in the opinion of the Investigator or Medical Monitor could compromise the ability of the patient to cooperate with study visits and procedures.

11. Use of any of the restricted list of products known to strongly inhibit cytochrome P450 (CYP) 3A4 drug metabolism (Appendix 15.4, Table 9) within 5 days prior to Day 1 dosing; or to strongly induce CYP3A4 metabolism (Appendix 15.4, Table 10) within 28 days prior to Day 1 dosing; or to strongly inhibit P-glycoprotein (P-gp) transporter (Appendix 15.4, Table 11) within 5 days prior to Day 1 dosing; or digoxin within 5 days prior to Day 1 dosing.

12. Serum bilirubin > ULN attributable to factors other than hemolysis and/or Gilbert's syndrome.

13. Male patients with heart-rate corrected QT interval -Fridericia's method (QTcF) interval > 450 msec, or female patients with QTcF interval > 470 msec with the exception of patients with a left bundle branch block (LBBB). Medical Monitor approval needed in patients with a LBBB.

14. Cardiac dysrhythmias judged as clinically significant by the Investigator or requiring therapy with drugs that are primarily substrates of CYP3A4.

15. History of allergy to sulfonamides if characterized by acute hemolytic anemia, anaphylaxis, or

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rash of erythema multiforme type or Stevens-Johnson syndrome.

16. Any other medical or psychological condition deemed by the Investigator to be likely to interfere with a patient's ability to understand and sign informed consent; cooperate with study visits, tests, and procedures; or otherwise safely and reliably participate in the study.

Patients will not be permitted to enter the Extension Period if:

17. The patient experienced AEs during the Core Period that are considered by the treating Investigator or the Sponsor's designated Medical Monitor or Responsible Medical Officer to pose a significant safety risk to the patient if treatment were to be extended.

**Investigational product, dosage, and mode of administration:**
AG-348 sulfate hydrate capsules will be provided as 5 mg, 25 mg, or 100 mg (free-base equivalent) of AG-348 sulfate hydrate without excipients in dark green opaque (5 mg), Swedish orange (25 mg), or white opaque (100 mg) gelatin capsules (size 2 capsules for all dose strengths). AG-348 will be administered PO BID. The number of capsules per dose will vary by assigned dose group. AG-348 will be administered with water and may be administered with or without food.

**Reference therapy, dosage and mode of administration:**
Not applicable.

**Duration of treatment:**
The duration of treatment for all patients in the Core Period will be up to 24 weeks. Patients who safely tolerate AG-348 and for whom the Investigator agrees with continuation of AG-348 treatment may be eligible to immediately roll over to the Extension Period for continued treatment (up to 2 years following completion of the Core Period).

**Criteria for evaluation:**

**Safety:**

Monitoring of AEs in randomized patients, including determination of serious AEs (SAEs) and AEs leading to discontinuation; safety laboratory parameters (hematology, chemistry, urinalysis, coagulation); physical examination findings (including neurological examination); VS; 12-lead ECGs, and DXA scans. Adverse events will be graded using CTCAE, Version 4.03. Serum sex hormone levels (testosterone [total and free], estrone, and estradiol), bone turnover markers (serum osteocalcin-N-mid and serum C-terminal telopeptide [CTX]), 25-hydroxy vitamin D2 and D3, total cholesterol, high-density lipoprotein-cholesterol (HDL-C), and triglycerides will be monitored for evidence of potential inhibition of aromatase by AG-348. Menstruating female patients will also keep a paper-based menstrual cycle diary throughout the study.

**Indicators of Clinical Activity:**

Monitoring of potential indicators of clinical activity will include evaluating changes in Hb, HCT, reticulocyte count, Hp, COHb, ETCO (Core Period only), LDH, total and indirect bilirubin, EPO, hepcidin, ferritin, and transferrin saturation.

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**Pharmacokinetics:**

Approximately the first 10 patients treated in the Core Period, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 15.1, Table 7. The remainder of treated patients will undergo limited PK sampling as detailed in Appendix 15.1, Table 8. During the Core Period, serial blood sampling for determination of concentration-time profiles of AG-348 and its metabolite AGI-8702 will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of AG-348 and AGI-8702 will be obtained. During the Extension Period, predose PK samples will be drawn for the measurement of trough levels of AG-348 and AGI-8702 at each study visit (every 3 months; see Appendix 15.1, Table 6). AG-348 and AGI-8702 will be analyzed using qualified assays to determine concentrations in plasma. Pharmacokinetic parameters on Day 1 and Day 15 will be computed using standard non-compartmental methods based on observed plasma AG-348 and AGI-8702 concentrations.

**Pharmacodynamics:**

Pharmacodynamic assessments during the Core Period will include 2,3-DPG, ATP (secondary objectives), [redacted]

Approximately the first 10 patients treated during the Core Period will undergo extensive PD sampling as detailed in Appendix 15.1, Table 7. The remainder of treated patients will undergo limited PD sampling as detailed in Appendix 15.1, Table 8. During the Core Period, serial blood sampling for determination of levels of ATP and, 2,3-DPG will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of ATP and 2,3-DPG will be obtained. During the Extension Period, predose PD samples will be drawn for the measurement of trough levels of 2,3-DPG, ATP, [redacted] at each study visit (every 3 months; see Appendix 15.1, Table 6). Adenosine triphosphate and 2,3 DPG will be analyzed using qualified assays to determine concentrations in whole blood. Pharmacodynamic parameters on Day 1 and Day 15 will be computed based on observed whole blood ATP and 2,3-DPG concentrations.

[redacted]

**Statistical methods:**

The primary objective during the Core Period of this Phase 2 study is to evaluate the safety and tolerability of up to 24 weeks of AG-348 in patients with PK deficiency. Therefore, analyses will be primarily descriptive in nature; no formal hypothesis testing will be conducted. All analyses will be

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conducted separately within each dose arm, or pooled when appropriate Analyses of safety and of indicators of clinical activity will be conducted for the Core Period, Extension Period, and overall, if applicable. For the Core Period, the data to be analyzed will include all collected data through 24 weeks of treatment for patients who directly enter the Extension period. For patients who do not enter the Extension Period, the analyses will include all collected data through the duration of treatment (24 weeks or less) plus 4 week follow up data. For patients who move directly from the Core to the Extension Period, the 4 week follow up data will be analyzed as part of the Extension Period.

Summaries will be produced for disposition, baseline disease characteristics and demographic data including genotype, safety measurements, PK, PD parameters and indicators of clinical activity. Data from each AG-348 dose group will be analyzed separately, and pooled across all dose groups where appropriate. All data will also be listed by individual patient. Categorical variables will be summarized by frequency distributions (number and percentages) and continuous variables will be summarized by descriptive statistics (mean, standard deviation [SD], median, minimum, and maximum).

Populations for analysis will include a Safety Analysis Set, a PK Analysis Set, and an Efficacy Analysis Set. The Safety Analysis set will include all patients who are enrolled and receive any dose of study treatment; this population will be the primary set for the analysis of safety data and the default analysis set for all data analyses unless otherwise stated. Patients will be classified according to treatment received, where treatment received is defined as the assigned treatment if it is received at least once, or as the first treatment received if assigned treatment is never received. The PK Analysis Set will include all patients in the Safety Analysis Set with sufficient plasma sample data to assess PK parameters. Results of the potential PD activity of AG-348 will also be based on the PK analysis set. The Efficacy Analysis Set will include all patients who are enrolled and achieve at least 50% compliance with their assigned dose intensity for at least 4 weeks of continued dosing. Additional efficacy analyses may be performed on subsets of patients with other degrees of compliance with their assigned dose intensity and/or duration of dosing. The Efficacy Analysis Set will be the primary set for the analysis of preliminary clinical activity data. Patients will be classified according to assigned treatment. [redacted]

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and the incidence of treatment-emergent AEs (TEAEs) (new or worsening from baseline) will be summarized by primary system organ class (SOC), preferred term, CTCAE Version 4.03 severity, outcome, action taken with study drug, and relationship to study drug by dose group. Separate summaries will be produced for all TEAEs, treatment-related AEs (those considered by the Investigator as at least possibly drug related), SAEs, discontinuations due to AEs, and AEs ≥ Grade 3 severity. Individual patient listings will be provided for any deaths, SAEs, AEs leading to interruption and/or reduction of study drug dose, and AEs leading to discontinuation of treatment. Analyses of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined as any AE with onset beginning at the day of first administration of study drug, throughout the treatment period until 30 days after the last dose of study drug, or any event that was present at baseline but worsened in intensity or was subsequently considered drug-related by the Investigator through the end of the study.

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Descriptive statistics will be provided for clinical laboratory values (e.g., hematology, serum chemistry, coagulation studies, urinalysis) and VS data, presented as both actual values and changes from baseline relative to each on-study evaluation. Shift analyses will be conducted for selected laboratory parameters based on the baseline CTCAE grade to maximum CTCAE grade. Where applicable CTCAE terms do not exist, a grading system based on the upper and/or lower limits of normal will be used to classify the results.

Electrocardiogram analyses will include individual patient listings and summaries of abnormal and clinically significant ECG results. Actual values and changes from baseline in the portion of the ECG wave from the beginning of the P wave to the beginning of the QRS complex (PR), QRS, heart rate-corrected QT intervals (QTc) will be summarized by visit and dose arm.

Data collected from the menstrual diaries such as the start and stop dates of the menses and the patient reported characteristics of the menses will be presented in a by-patient listing. Additional descriptions of the data may also be performed.

Concomitant medications will be coded using the WHO Drug Dictionary. Concomitant medications prior to and after the start of the study drug will be listed by patient, and summarized by ATC term and dose arm.

Descriptive statistics will be used to summarize PK parameters for the parent compound AG-348 and the metabolite AGI-8702 for each dose group, and where appropriate, for the entire population. Pharmacokinetic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation.

Descriptive statistics will be used to summarize PD parameters for 2,3-DPG and ATP for each dose group, and where appropriate for the entire population. Pharmacodynamic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation %.

Analyses of serum sex hormones will use appropriate graphic displays and statistical analyses to evaluate patient change from baseline for each parameter. These analyses will include summaries of actual values and change from baseline using appropriate descriptive statistics (mean, SD, median, min and max) as described above.

Analyses evaluating indicators of potential clinical activity of AG-348 in patients with PK deficiency will include changes in Hb, HCT, reticulocyte count, Hp, COHb, ETCO (Core Period only), LDH, total and indirect bilirubin, EPO, hepcidin, ferritin, and transferrin saturation (serum iron/iron binding capacity). Characterization of Hb response (e.g., % of patients whose Hb increases by a certain amount), as well as time to Hb response, and duration of Hb response will be explored, among others.

The study database will be locked and statistical analysis will be performed after all patients have completed the Core Period. The results of this analysis will be presented in a clinical study report (CSR). Additional data collected during the Extension Period after the CSR database lock will be analyzed for inclusion in a subsequent CSR addendum.

**Interim Review**

No formal statistical analysis will be conducted. Safety data will be reviewed on an ongoing basis by the

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| DRT, who will meet to review safety, PK, PD, and preliminary clinical activity data at regular intervals (approximately every 6 weeks during the Core Period and approximately every 3 months during the Extension Period once all patients have completed the Core Period) throughout the duration of the study. The composition of the DRT, its meeting schedule, the data to be reviewed, and the decisions it is empowered to make have been described previously.<br>Additional interim reviews of data may be conducted to support decision making concerning the current clinical study, the sponsor's development programs in general, or in case of any safety concerns.<br><br>**Sample Size**<br>Due to the rare disease setting, the minimal sample size may be determined by feasibility. Depending on possible early termination of 1 or both of the initial 2 arms or the addition of a 3rd dose arm, the study could enroll up to a maximum of 75 patients. |

# 3. TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

## TABLE OF CONTENTS

## LIST OF TABLES



## LIST OF FIGURES

# 4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

| Abbreviation or Specialist Term | Explanation |
|---|---|
| 2,3-DPG | 2,3-diphosphoglycerate |
| ACIP | Advisory Committee on Immunization Practices |
| ADP | Adenosine diphosphate |
| AE | Adverse event |
| ALC | Absolute lymphocyte count |
| ALP | Alkaline phosphatase |
| ALT | Alanine aminotransferase |
| ANC | Absolute neutrophil count |
| aPTT | Activated partial thromboplastin time |
| AST | Aspartate aminotransferase |
| ATP | Adenosine triphosphate |
| AUC | Area under the curve |
| $AUC_{0\text{-}12hr}$ | Area under the plasma concentration versus time curve from 0 to 12 hours |
| $AUC_{0\ 24hr}$ | Area under the plasma concentration versus time curve from 0 to 24 hours |
| $AUC_{0\text{-}\infty}$ | Area under the plasma concentration versus time curve from 0 to infinity |
| BCRP | Breast cancer resistance protein |
| BID | Twice daily |
| BP | Blood pressure |
| BUN | Blood urea nitrogen |
| CBC | Complete blood count |
| CDC | Centers for Disease Control and Prevention |
| $C_{max}$ | Maximum plasma concentration |
| $CO_2$ | Carbon dioxide |
| COHb | Carboxyhemoglobin |
| CSR | Clinical study report |
| CTCAE | Common Terminology Criteria for Adverse Events |
| CTX | Serum C-terminal telopeptide |
| CV | Cardiovascular |

| Abbreviation or Specialist Term | Explanation |
|---|---|
| DDI | Drug-drug interaction |
| CYP | Cytochrome P450 |
| DILI | Drug-induced liver injury |
| DLT | Dose-limiting toxicity |
| DRT | Data review team |
| DXA | Dual-energy x-ray absorptiometry |
| ECG | Electrocardiogram |
| ECOG | Eastern Cooperative Oncology Group |
| eCRF | Electronic case report form |
| EDC | Electronic Data Capture |
| EPO | Erythropoietin |
| ETCO | End tidal carbon monoxide |
| FDA | Food and Drug Administration |
| FSH | Follicle stimulating hormone |
| G6PD | Glucose-6-phosphate-dehydrogenase |
| GCP | Good Clinical Practice |
| GFR | Glomerular filtration rate |
| GLP | Good Laboratory Practice |
| Hb | Hemoglobin |
| HBsAg | Hepatitis B surface antigen |
| HCT | Hematocrit |
| HCV | Hepatitis C virus |
| HDL-C | High-density lipoprotein-C |
| HIV | Human immunodeficiency virus |
| Hp | Haptoglobin |
| $IC_{50}$ | Concentration of drug that achieved half-maximal inhibition |
| ICH | International Conference on Harmonization |
| IEC | Independent Ethics Committee |
| INR | International normalized ratio |
| IRB | Institutional Review Board |
| IUD | Intrauterine device |
| LBBB | Left bundle branch block |

| Abbreviation or Specialist Term | Explanation |
|---|---|
| LDH | Lactate dehydrogenase |
| MAD | Multiple ascending dose |
| MCH | Mean corpuscular hemoglobin |
| MCHC | Mean corpuscular hemoglobin concentration |
| MCV | Mean corpuscular volume |
| MedDRA | Medical Dictionary for Regulatory Activities |
| mPKR | PKR mutants |
| MTD | Maximum tolerated dose |
| NCI | National Cancer Institute |
| NOEL | No-observed-effect-level |
| NOAEL | No-observed-adverse-effect-level |
| P-gp | P-glycoprotein |
| PCV13 | Pneumococcal Conjugate |
| PD | Pharmacodynamic |
| PEP | Phosphoenolpyruvate |
| PK | Pharmacokinetic |
| PK deficiency | Pyruvate kinase deficiency |
| PKM2 | Pyruvate kinase isoform M2 |
| PKR | Pyruvate kinase isoform R |
| PO | Oral(ly) |
| PPSV23 | Pneumococcal polysaccharide |
| PR | The portion of the ECG wave from the beginning of the P wave to the beginning of the QRS complex |
| q12h | Every 12 hours |
| q24h | Every 24 hours |
| QD | Once-daily |
| QTc | Heart-rate corrected QT interval |
| QTcB | Corrected QT interval - Bazett correction formula |
| QTcF | Corrected QT interval - Fridericia's method |
| RBC | Red blood cell (count) |
| RDW | Red cell distribution width |
| SAD | Single ascending dose |
| SAE | Serious adverse event |

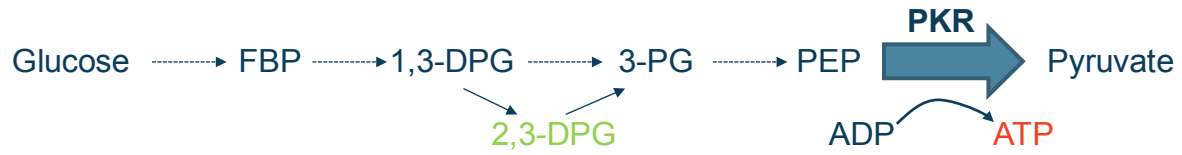
| Abbreviation or Specialist Term | Explanation |
|---|---|
| SAP | Statistical analysis plan |
| SD | Standard deviation |
| SOC | System organ class |
| $t_{½}$ | Apparent terminal half-life |
| TIBC | Total iron-binding capacity |
| $T_{max}$ | Time to maximum plasma concentration |
| TEAE | Treatment-emergent adverse event |
| ULN | Upper limit of normal |
| VS | Vital signs |
| $V_{ss}$ | Volume of distribution at steady-state |
| Vz/F | Mean apparent volume of distribution |
| WBC | White blood cell |
| WMA | World Medical Association |
| WOCBP | Women of childbearing potential |
| WT | Wild type |

# 5. INTRODUCTION

## 5.1. Pyruvate Kinase Deficiency

Pyruvate kinase deficiency (PK deficiency) is a glycolytic enzymopathy that results in non-spherocytic hemolytic anemia with a variable clinical presentation, ranging from mild or fully compensated forms to life-threatening neonatal anemia and life-long chronic hemolytic anemia associated with severe, debilitating co-morbidities. Pyruvate kinase deficiency is caused by mutations in the PKLR gene, which results in defective pyruvate kinase isoform R (PKR) in the red blood cell (RBC). As shown in the diagram below, PKR catalyzes the final and irreversible step in glycolysis, converting phosphoenolpyruvate (PEP) to pyruvate, and formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP). Mature RBCs rely almost exclusively on the process of glycolysis to generate the energy carrier molecule ATP. PKR is thus a key enzyme for maintaining energy homeostasis in erythrocytes, and it has been proposed that ATP levels are critical for optimally maintaining RBC membrane integrity (van Wijk and van Solinge 2005).

**Figure 2: Red Cell Glycolysis**




Abbreviations: 1,3 DPG = 1,3 diphosphoglycerate; 2,3 DPG = 2,3 diphosphoglycerate; 3-PG = 3-phosphoglycerate; ADP = adenosine diphosphate; ATP = adenosine triphosphate; FBP = fructose 1,6-bisphosphate; PEP = phosphoenolpyruvate; PKR = red blood cell-specific form of pyruvate kinase
Note: Not all steps in glycolysis are shown.

The majority of mutations in PKR that have been described have a deleterious effect on PKR catalytic activity, protein stability, and/or protein expression. Pyruvate kinase deficiency is associated with reduced RBC survival as well as impaired red cell maturation. Mature erythrocytes lack mitochondria relying predominantly on glycolysis to generate ATP. It is hypothesized that insufficient energy production promotes erythrocyte hemolysis due to impaired maintenance of cellular membrane homeostasis.

Pyruvate kinase deficiency is an autosomal recessive disease, in which both homozygotes and compound heterozygotes develop hemolytic anemia. More than 200 different mutations in the PKLR gene have been identified in patients with PK deficiency to date, and the majority of these mutations are single nucleotide missense mutations (Zanella, et al. 2005). The prevalence of PK deficiency, based on the most common pyruvate kinase mutation in Southern Europe, is estimated at 51 cases per million in the Caucasian population (Beutler and Gelbart 2000).

Some patients with PK deficiency have a clinically severe disease course that can present as a severe hemolytic anemia in early infancy, requiring immediate care which typically includes blood transfusion support (Christensen, et al. 2011; Ghidini, et al. 1991; Hennekam, et al. 1990). Infants with PK deficiency may have erythroblastosis fetalis with hepatosplenomegaly, extramedullary hematopoiesis, and severe indirect hyperbilirubinemia, which is associated with a

high risk of kernicterus. Hyperbilirubinemia associated with the hemolytic state often requires supportive treatment with intensive phototherapy. For other infants, the hyperbilirubinemia caused by hemolysis can require exchange transfusion, as well as supportive transfusion to keep their hematocrit (HCT) levels >60%.

Adults with PK deficiency are reported to have life-long hemolysis and the subsequent associated co-morbidities including anemia and transfusion dependence. Other co-morbidities include frequent miscarriages, aplastic crises, as well as symptoms associated with an acute on chronic hemolytic anemia (Rider, et al. 2011). Since unconjugated bilirubin is often chronically elevated, pigmented gallstones are common in children and adults. Severe and sometimes life-threatening iron deposition occurs and is typically progressive.

The current therapeutic options for patients with PK deficiency are supportive. Most require life-long treatment, including blood transfusions at a frequency depending on the disease state. Long-term surveillance for systemic iron overload, even in transfusion-independent patients, is standard as is the use of chelation therapy. Case reports of cure by allogeneic bone marrow transplant have been published (Tanphaichitr, et al. 2000) but have been infrequently performed. Splenectomy, while not curative, can reduce the need for supportive blood transfusions and decrease bilirubin levels in some patients. However, this intervention is associated with significant morbidity including infection risk, and possible increased risk of thrombosis.

## 5.2. AG-348

AG-348 is an orally available, allosteric activator of PKR. It is hypothesized that drug intervention with AG-348 restores glycolytic pathway activity and normalizes red cell metabolism *in vivo*. Biochemical experiments demonstrate that AG-348 is a potent, pan-activator of many PKR alleles associated with PK deficiency. Pyruvate kinsase deficiency red cells and their progenitors are characterized by changes in metabolism associated with defective glycolysis, including a build-up of PEP and 2,3-diphosphoglycerate (2,3-DPG), and lowered ATP levels. Treatment of PK deficiency patient red cells *ex vivo* with AG-348 results in increased ATP levels, and reductions in PEP and 2,3-DPG, consistent with pharmacological activation of PKR enzyme activity. *In vitro* studies against wild type (WT) PKR enzymes and *ex vivo* studies in blood from mice, monkeys and humans indicate that AG-348 activates WT PKR with a similar potency and efficacy as it does with the mutant versions. *In vivo* studies in mice confirm the potency of AG-348 in increasing WT PKR enzyme activity and modulating the levels of downstream markers such as ATP and 2,3-DPG. This therapeutic approach may be an effective way to correct the underlying pathology of PK deficiency and, importantly, provide clinical benefit to patients.

### 5.2.1. Summary of Nonclinical Data

A series of exploratory pharmacology studies were conducted to characterize the ability of AG-348 to activate WT PKR and anemia-associated PKR mutants (mPKR) *in vitro*, *ex vivo*, and *in vivo*. Additional details regarding nonclinical data can be found in the AG-348 Investigator's Brochure

#### 5.2.1.1. Pharmacology

AG-348 is a potent, broad-spectrum activator of PKR with low nM potency against both WT and mutant enzymes. AG-348 is also able to effectively stabilize mPKR enzymes that are hypersensitive to thermal denaturation, suggesting that AG-348 may be able to increase PKR pathway activity by preventing destabilization and degradation of mPKR enzymes. Its major metabolite, AGI-8702, has low potency mixed activity against PKR and mutant isoforms and can increase the thermostability of some mPKR, albeit with significantly weaker potency than that of AG-348.

The effect of AG-348 and AGI-8702 on PKR activity and a number of downstream pathway markers was evaluated in human RBCs. AG-348 dose-response curves in human whole blood showed potent activation of PKR, while AGI-8702 is a weak activator of the PKR enzyme. In whole blood from mice treated with AG-348, $^{13}$C-glucose labeling through the PKR reaction was increased by 80% demonstrating AG-348-mediated activation of the WT PKR enzyme. AG-348 treatment of RBCs from healthy human donors potently increased levels of the PKR reaction product ATP consistent with activation of PKR.

The effects of AG-348 on PKR activity and RBC metabolism also were assessed in blood samples from patients with PK deficiency. AG-348 activated PKR and induced metabolic changes (increased ATP levels and decreased 2,3-DPG levels) consistent with increased glycolytic pathway activity in RBCs from PK deficiency patients with different mutations in the PKR enzyme.

Assessments of binding inhibition potential and enzymatic activity were evaluated in a panel of 89 receptors, ion channels, and enzymes. Although AG-348 is a histamine H3 antagonist/inverse agonist and an aromatase inhibitor, no effects of histamine H3 modulation have been observed in safety pharmacology or toxicology studies. Effects consistent with aromatase inhibition were seen in toxicology studies (Section 5.2.1.4).

#### 5.2.1.2. Safety Pharmacology

Safety pharmacology studies demonstrated that neither AG-348 nor AGI-8702 exhibited significant inhibition of the human ether-à-go-go related gene (hERG) current (concentration of drug that achieved half-maximal inhibition [$IC_{50}$] 29.4 µM and > 10 µM, respectively). Additionally, in the monkey cardiovascular (CV) study, no CV effects were seen at any dose level. No effects were seen in AG-348 Good Laboratory Practice (GLP)-compliant Irwin and respiratory safety pharmacology studies in Sprague-Dawley rats. AG-348 administered via oral gavage had a dose-dependent emetic activity in the ferret at ≥ 30 mg/kg.

#### 5.2.1.3. Pharmacokinetics

Absorption, distribution, metabolism, and excretion (ADME) studies of AG-348 were performed in Sprague-Dawley rats, beagle dogs, and cynomolgus monkeys. Both oral (PO) and intravenous (IV) routes were evaluated at doses ranging from 1 to 50 mg/kg. A dose of 200 mg (50 mg/kg) was administered PO in the food-effect evaluation in monkeys. The PK of AG-348 in animal species is characterized by rapid oral absorption, medium to high total body plasma clearance (CLp), and high volume of distribution at steady-state ($V_{ss}$) in rats, dogs, and monkeys.

AG-348 has a medium to long apparent terminal half-life ($t_{1/2}$) in the rat, dog, and monkey. Medium to high oral bioavailability was observed for AG-348 in animals. A high-fat diet led to lower oral absorption of AG-348 in monkeys.

AG-348 showed low brain penetration in rats (brain/plasma ratio of 5.7% to 9.1%) based on area under the plasma concentration versus time curve from 0 to 24 hours ($AUC_{0\ 24hr}$) following single and repeated-dose administration, respectively. Metabolism appears to be the major elimination pathway for AG-348 with minimal biliary or urinary excretion.

Both *in vitro* and *in vivo*, the predominant metabolite was the N-dealkylated product, AGI-8702. There were no metabolites unique to human liver microsomes and no direct Phase 2 metabolites. The plasma metabolite profile of AG-348 did not change with repeat or ascending dosing, suggesting that AG-348 is not susceptible to metabolic switching with increasing or prolonged dose administration.

AG-348 is a weak direct inhibitor of cytochrome P450 (CYP) 2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 (testosterone 6β-hydroxylation). There was no direct inhibition of CYP1A2, CYP2B6, or CYP3A4/5 (midazolam 1′-hydroxylation) by AG-348. There was evidence of metabolism-dependent inhibition of CYP2C19 (largely reversible) and CYP3A4 (largely irreversible) by AG-348. AG-348 is considered a weak time-dependent CYP3A4 inhibitor. AGI-8702 is a weak direct inhibitor of CYP2C9 and CYP2C19. AG-348 has the potential to cause an induction-related drug-drug interaction (DDI) with sensitive CYP2B6 and CYP3A4 substrates.

The routes of metabolism for AG-348 are via multiple CYPs with CYP3A4 contributing > 70% of the total. CYP1A2, CYP2C9, and CYP2C8 contribute approximately 6%, 10%, and 7% to the remaining metabolism of AG-348; other isoforms contribute < 4% each.

AG-348 is a substrate for P-glycoprotein (P-gp), but not breast cancer resistance protein (BCRP). AG-348 is an inhibitor of P-gp (91% and 99% inhibition at 41 and 411 μM, respectively), but does not inhibit BCRP. AGI-8702 is not an inhibitor of P-gp or BCRP under the concentrations tested (5 and 100 μM).

#### 5.2.1.4. Toxicology

In rats, the no-observed-effect-level/no-observed-adverse-effect-level (NOEL/NOAEL), determined as 2000 mg/kg, was associated with area under the plasma concentration versus time curve from 0 to 12 hours ($AUC_{0\text{-}12hr}$) values 223- to 526-fold the projected human efficacious $AUC_{0\text{-}12hr}$ value. In dogs, clinical observations consistent with anaphylactoid reactions were seen, and the maximum tolerated dose (MTD) was 62.5 mg/kg, which was associated with an $AUC_{0\text{-}12hr}$ value 5.7-fold the projected efficacious $AUC_{0\text{-}12hr}$ value. The NOEL/NOAEL in dogs was 10 mg/kg, which was associated with an $AUC_{0\text{-}12hr}$ value 0.8-fold the projected efficacious $AUC_{0\text{-}12hr}$ value. In monkeys, the NOAEL was 1000 mg/kg, with only non-adverse emesis and body weight loss seen; this dose was associated with an $AUC_{0\text{-}12hr}$ value 70-fold the projected efficacious $AUC_{0\ 12hr}$ value. Based on the results of single-dose studies, the rat and the monkey were chosen as the most appropriate species for further evaluation in toxicology studies.

Dose-limiting toxicity (DLT) in cynomolgus monkeys was defined in non-GLP 5-day and 14-day repeat-dose studies as emesis, inappetence, and weight loss. These toxicities became dose limiting at $AUC_{0\text{-}12hr}$ values 27- to 34-fold the projected efficacious $AUC_{0\text{-}12hr}$ value, and

precluded meaningful evaluation of other toxicities at this exposure level. Potential other effects at this exposure level were observed in a number hematology and serum chemistry parameters as well as in lymphoid organs. Additionally, minimal potential effects in kidneys (renal tubulointerstitial nephritis) and heart (myocardial degeneration), which could not be differentiated from spontaneous background lesions, were seen when monkeys were exposed to AG-348 $AUC_{0\text{-}12hr}$ values ≥ 27- to 34-fold the projected human efficacious $AUC_{0\text{-}12hr}$ value.

In GLP-compliant 28-day monkey study, the dose of 150 mg/kg/day (75 mg/kg/dose twice daily [BID]) was the NOAEL. Effects were limited to increased liver weights without serum chemistry or microscopic correlate. At this dosage level, the Day 27 $AUC_{0\text{-}12hr}$ values were 8.9- and 8.5-fold the projected efficacious $AUC_{0\text{-}12hr}$ value in males and females respectively. In the same study, the low dosage of 20 mg/kg/day (10 mg/kg/dose) resulted in $AUC_{0\text{-}12hr}$ values that approximated the efficacious $AUC_{0\text{-}12hr}$ value, and there were no test article-related effects seen. The next highest dose of 50 mg/kg/day (25 mg/kg/dose) was the NOEL and was associated with $AUC_{0\text{-}12hr}$ values 3.1- and 2.6-fold the projected efficacious $AUC_{0\text{-}12hr}$ value in males and females respectively.

In the GLP-compliant 28-day rat study, the NOAEL in males was the highest dose tested, 600 mg/kg/day (300 mg/kg/dose), and the NOAEL in females was the lowest dose tested, 20 mg/kg/day (10 mg/kg/dose). At the 600 mg/kg/day dosage level in males, AG-348-related findings were limited to mild effects on hematology, serum chemistry, and urinalysis parameters, and microscopic findings in the adrenal gland (minimal to mild vacuolation of the adrenal zona glomerulosa and decreased thickness of the zona fasciculate), liver (minimal to mild hepatocellular hypertrophy), kidney (minimal tubular vacuolation), pancreas (minimal to moderate decreased zymogen granules), heart (minimal myocardial vacuolation), and prostate (minimal to mild decreased secretion). All findings were fully reversible over the 14-day recovery period with the exception of decreased serum glucose levels and decreased prostate secretion. In females, the highest dosage tested was 200 mg/kg/day (100 mg/kg/dose); adverse effects observed were similar to those observed in the 600 mg/kg/day males, with the exception that in females, fewer effects in hematology and serum chemistry parameters were seen, and also in females, adverse effects in the reproductive organs consistent with aromatase inhibition were observed.

In the 13-week repeat-dose rat study, adverse effects in males were identified in the testes and consisted of seminiferous tubular degeneration, spermatid retention, and Leydig cell hypertrophy. These effects were defined as adverse at dose levels at which they are expected to impair fertility. These effects were either not present or present with incidence and severity similar to that of the vehicle group in lower dose levels. Adverse effects in females included uterine atrophy and increased folding of the luminal surface; these effects were defined as adverse at the dose level at which they are expected to impair fertility

In the 13-week repeat-dose study in monkeys, no adverse effects were identified, and no new effects were identified when compared to the 4-week repeat-dose study. Similar to what occurred on the 4-week study, inappetence and emesis during the initial 1-2 weeks of dosing occurred, precluding evaluation of higher doses.

AG-348 inhibits human aromatase activity with an $IC_{50}$ of 2050 nM (based on human placental microsomes) and rat aromatase with an $IC_{50}$ of 493 nM (based on rat ovarian microsomes). In the GLP-compliant 28-day rat study, histologic effects consistent with aromatase inhibition were

seen in the female reproductive tract at the mid- and high-dosage levels (100 and 200 mg/kg/day) and included incomplete corpora lutea; ovarian follicular cysts; ovarian cystic, luteinized follicles; uterine atrophy; vaginal mucification; and altered cyclicity. Although these findings were minimal to mild and were fully reversible (over 14 days), they were considered adverse and the next lower dosage evaluated, 20 mg/kg/day (10 mg/kg/dose BID) was the NOAEL in females. The Day 27 $AUC_{0\text{-}12hr}$ value associated with this dosage level was 6.9-fold the projected human efficacious $AUC_{0\text{-}12hr}$ value. The potential for aromatase inhibition effects occurring in female rats at $AUC_{0\text{-}12hr}$ values > 6.9-fold and < 53-fold the projected efficacious $AUC_{0\text{-}12hr}$ value has been addressed in a 13-week rat study. In this study using doses between the NOAEL and LOAEL in the 28-day study, the NOAEL for histologic lesions of the uterus that may be associated with aromatase inhibition resulted in an $AUC_{0\text{-}12hr}$ that was 26-fold the projected efficacious value. Notably, due to the potency difference of AG-348 against rat versus human aromatase inhibition, there is potential for a 4-fold wider margin for aromatase inhibition in humans versus rats. AGI-8702 is not an aromatase inhibitor.

### 5.2.2. Summary of Clinical Data

To date, 72 healthy adult volunteers have been exposed to AG-348 in 2 clinical studies, a single ascending dose (SAD) study and a multiple ascending dose (MAD) study, with 31 of these subjects exposed to a single AG-348 dose under fasted conditions; 5 exposed to 2 doses of AG-348 under fasted and fed conditions; and 36 exposed to repeated doses of AG-348 for up to 14 days. Except as otherwise specified, the following discussion of clinical data refers only to healthy adult volunteer subjects, as this is the first clinical trial in which patients with PK deficiency will be treated with AG-348.

#### 5.2.2.1. Pharmacokinetics

The pharmacokinetics (PK) of AG-348 showed low to moderate variability between subjects. After single doses of AG-348 from 30 to 2500 mg, AG-348 was rapidly absorbed as indicated by the short time to maximum plasma concentration ($T_{max}$). There appeared to be some lengthening of the period of absorption at higher doses as indicated by higher $T_{max}$ and a less than proportional increase in maximum plasma concentration ($C_{max}$). Dose-normalized area under the curve (AUC) generally remained constant over the dose range studied, suggesting that AG-348 total exposure increased in a dose-proportional manner. The mean $t_{1/2}$ ranged from 17.8 to 20.4 hours when samples were collected through 72 hours and from 50.3 to 79.3 when samples were collected through 120 hours. However, this terminal elimination phase contributed little to overall exposure of AG-348, as indicated by the small difference between $AUC_{0\text{-}12}$ and area under the plasma concentration versus time curve from 0 to infinity ($AUC_{0\text{-}\infty}$), suggesting a shorter effective half-life of approximately 3 to 6 hours. AG-348 was extensively distributed (mean apparent volume of distribution [$V_z/F$] range of 271 to 1148 L) and had a moderate rate of clearance (geometric mean clearance [CL/F] range of 10.3 to 13.9 L/hr). The fraction of AG-348 excreted in urine ranged from 0.0145 to 0.0209 across the dose levels suggesting that renal excretion plays a minor role in the systemic elimination of AG-348.

The preliminary repeat-dose PK of AG-348 at doses ranging from 15 mg every 12 hours (q12h) to 700 mg q12h also showed an increase in AG-348 exposure in a dose-proportional manner after the first dose. AG-348 exposure observed after repeated dosing for 14 days at doses of 120 mg every 24 hours (q24h) to 700 mg q12h were lower than observed on Day 1 after the first

dose. The magnitude of the effect was greater at higher doses; lower doses of 15 mg q12h and 60 mg q12h did not show this effect. This observation may be related to the nonclinical finding that AG-348 is a potential inducer of human CYP3A4, an enzyme which biotransforms AG-348. It is possible that multiple doses of AG-348 leads to an increased rate of its own metabolism.

Evaluation of the effect of food on the PK of a single 700 mg dose of AG-348 in 5 subjects who were administered the drug fasting and then, after an appropriate wash-out period, re-administered the drug following ingestion of a standard US Food and Drug Administration (FDA) high fat meal, showed that food likely has a minimal effect on the PK of AG-348.

#### 5.2.2.2. Pharmacodynamics

After a single dose of AG-348, a decrease in the concentration of 2,3-DPG was observed at 3 hours post-dose, decreased in a dose-dependent manner to a minimum at 24-hour post-dose, and then returned to values similar to baseline by 72 to 120 hours post-dose. The mean decrease at 24 hours was approximately 300 µg/mL at the 700 through 2500 mg dose levels. Similar decreases were observed after the first dose of multiple doses and prior to doses in the MAD study. After the final dose of multiple doses, the concentration of 2,3-DPG returned to values similar to baseline between 72 and 120 hours post- dose.

After a single dose of AG-348, a minimal increase in the concentration of ATP was observed at 24 to 120 hours post-dose. In the MAD study, no increase was observed at 12 hours after the first dose; the concentration of ATP was increased on Day 8 to concentrations greater than in the single dose study, continued to trend upward on Day 11, and remained at a similar level through Day 14. The concentration of ATP remained elevated through 120 hours after the last dose on Day 14. The magnitude of the increase in ATP was similar across the dose range from 60 mg BID to 700 mg BID.

#### 5.2.2.3. Safety

Overall, AG-348 has been well-tolerated among healthy volunteers at doses that produced strong pharmacodynamic (PD) effects on 2,3-DPG and ATP.

After a single AG-348 dose, treatment-emergent adverse events (TEAEs) reported by > 1 subject at any time on study (either under fasted or fed conditions) included headache (22%), nausea (14%), and contact dermatitis and vomiting (each 6%). After repeated dosing of AG-348 for 14 days, TEAEs that occurred in > 5% of all AG-348-treated subjects across all cohorts included headache (13.9%), nausea (13.9%), vomiting (8.3%), decreased appetite (8.3%), feeling hot (8.3%), restlessness (8.3%), and dizziness, fatigue, vessel puncture site bruise, hyperhidrosis, dermatitis allergic, and drug eruption (5.6% each).

A dose-relationship was apparent with regard to the incidence of gastrointestinal events, primarily nausea and vomiting, with the incidence of such events increasing with increasing dose. Nausea and/or vomiting were observed only at doses ≥ 1400 mg in the single dose study and only at doses ≥ 700 BID in the MAD study. Nausea and vomiting were not observed at any dose ≤ 360 mg in either the single or multiple dose studies.

All but 1 TEAE reported to date has been mild or moderate (Grade 1 or 2) in intensity. The only Grade 3 TEAE was elevated liver transaminases (alanine aminotransferase [ALT]; aspartate aminotransferase [AST]) in a single subject in the MAD study treated with 700 mg AG-348 BID.

The event was considered to be possibly related to AG-348, was declared a DLT, and led to study drug discontinuation, following which the elevated liver transaminases resolved. Altogether, 3 of 6 subjects treated with AG-348 at 700 mg BID discontinued study drug in the MAD study: the subject described above with Grade 3 elevated transaminases, and 2 others who withdrew themselves for nausea and vomiting. A fourth subject treated with AG-348 at 60 mg BID was discontinued from the study for a drug eruption. No AG-348-treated subject discontinued in the SAD study due to an adverse event (AE).

No deaths or other serious AEs (SAEs) have been reported in any clinical study of AG-348. Furthermore, no DLTs were observed after a single administration of AG-348 at doses of 30 mg to 2500 mg or after repeat administration of AG-348 for 14 days at daily doses of 15 mg to 700 mg BID except for the event of Grade 3 elevated liver function tests described above.

Due to preclinical observations pertaining to the potential for inhibition of the aromatase enzyme (see Section 5.2.1.4), the AG348-C-002 multiple dose study included assessment of baseline and serial measures of free and total serum testosterone and serum estradiol and estrone.

In males treated with AG-348, compared with placebo-treated males, the aromatase-dependent hormone assessments demonstrated an increase in total and free testosterone mean serum concentrations and decreased concentrations of estradiol and estrone at all doses of AG-348, including the lowest dose of 15 mg q12h. Most of the increases in total and free testosterone remained within the reference range, except at the 360 mg q12h dose where the dose group means on Day 8 and Day 14 exceeded the ULN. Most of the estradiol concentrations observed in males remained within the reference range, but the mean male estrone concentrations dropped to the lower limit of quantification in all dose groups except 15 mg q12h. These changes in aromatase-dependent hormone levels in the male subjects treated with AG-348 are consistent with inhibition of human aromatase, and were reversible within 14 days upon cessation of dosing. The study did not enroll enough female subjects to draw any definitive conclusions regarding serial changes in aromatase-dependent hormones over time.

As of as this protocol amendment (Study AG348-C-003, Version 5.0), 1 patient with PK deficiency ([redacted] year-old [redacted] 1151C>T [T384M]/1529G>A [R510Q]) treated with 300 mg AG-348 BID experienced an increase in hemoglobin (Hb) from baseline [redacted] g/dL ([redacted] g/L) to [redacted] g/dL ([redacted] g/L) and a decrease in serum bilirubin at Day 17. The patient's treatment was suspended for 2 days, according to the dose modification guidance in Amendment 2, and then resumed at 100 mg BID on Day 19. The Hb level decreased to [redacted] g/dL ([redacted] g/L) by Day 21 (before increasing again upon resuming AG-348) and was associated with an increase in serum bilirubin and symptoms of nausea and headache that were similar to symptoms the patient had previously noted during hemolytic episodes. This patient's course suggests a robust Hb response to AG-348 followed by hemolysis upon suspension of the drug. The patient tolerated these symptoms and did not require hospitalization. See Section 9.7.2 for further guidance on dose modification for increases in Hb level.

## 5.3. Study Rationale

Study AG348-C-003 is the first study that will be conducted in patients with PK deficiency. This study is primarily intended to evaluate the safety and tolerability and potential indicators of clinical activity of AG-348 administered for up to 24 weeks. This study will also evaluate the PK profile of AG-348 and its metabolite AGI-8702, the PD responses in ATP and 2,3-DPG

following administration of AG-348, and the clinical activity of AG-348 in PK deficiency patients. Two previous double-blind, placebo-controlled clinical trials of AG-348 conducted in healthy adult male and female volunteers (AG348-C-001, a SAD study; and AG348-C-002, a MAD study) have established an acceptable safety and tolerability profile for AG-348 for up to 14 days of both once-daily (QD) and BID dosing at exposures that result in significant PD changes in whole blood levels of the glycolytic metabolites 2,3-DPG and ATP. Due to the potential for AG-348-mediated aromatase inhibition, dual-energy x-ray absorptiometry (DXA) scan (hip and spine) will be performed at Screening (if patient has not had prior DXA scan within 3 months of Day 1) to obtain T and Z scores. These data will serve as baseline measures of bone mineral density for all enrolling patients (see Section 7.1 for more details).

The target population of this study consists of adult males and females with a diagnosis of PK deficiency, who are anemic but non-transfusion dependent. Non-transfusion dependent patients are preferred for this study in order to reduce any potential confounding effect of transfusion therapies on evaluation of potential indicators of clinical activity and PD responses. The safety, tolerability, and PK/PD findings in this study will form the basis for subsequent clinical development of AG-348.

The Extension Period is offered in order to allow for longer study of AG-348 in patients who experience a benefit and tolerate the drug.

### 5.3.1. Summary of Overall Safety Management Plan

Measures to minimize the risks to patients enrolled in the Core and Extension Periods have been taken with respect to the following study design elements:

- The initial doses for Arms 1 and 2 have been selected on the basis of safety and tolerability observations already carefully made in two predecessor clinical trials in adult healthy male and female volunteers;
- The specified inclusion/exclusion criteria have been carefully considered to avoid enrollment of subjects for whom exposure to the study drug might pose a hazard;
- A designated Data Review Team (DRT) consisting of the overall study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer has been established to meet regularly at approximately 6-week intervals throughout the duration of the Core Period, and approximately every 3 months during the Extension Period once all patients have completed the Core Period to review the accumulating study data and will exercise options to suspend enrollment to one or both of the initial two study dose arms, discontinue enrollment to one or both of the initial two study dose arms, adjust the dose of patients in one or both of the initial two study arms, and/or implement one new study dose arm. If one new dosing arm is implemented in the Core Period, the dose selected will not exceed 360 mg BID, the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy volunteers. Group cohort stopping rules for terminating enrollment into an arm based on the severity (Common Terminology Criteria for Adverse Events [CTCAE]v4.03 grade) and frequency of AEs are defined;
- Dose modification and stopping rules are defined for individual patients;

- Guidance for permitted, prohibited, and cautionary concomitant medications is specified based on the estimated potential for DDIs from hepatic cytochrome enzyme interactions with AG-348.
- Due to the potential for AG-348-mediated aromatase inhibition, bone mineral density will be monitored using DXA scans (hip and spine) at Baseline (if patient has not had prior DXA scan within 3 months of Day 1) and between Week 24 and Week 28.

In the event that any clear and unequivocal, previously unidentified/unexpected toxicities occur in pre-clinical toxicology studies, the Sponsor will notify the Investigators, Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs), and regulatory authorities. The Sponsor will also report these to the DRT for review and discussion of appropriate actions, which may include, but are not necessarily limited to:

- Amending the protocol to adjust the inclusion/exclusion criteria (e.g., to exclude patients with certain at-risk concurrent conditions); and/or
- Amending the protocol to adjust safety monitoring procedures (e.g., to require additional monitoring of specified AEs, physical examinations, clinical laboratory testing, ECG monitoring, or other testing as appropriate); and/or
- Adjusting the dose of an arm of the study as appropriate; and/or
- Adjusting the dose modification and/or stopping rules (Section 9.7); and/or
- Adjusting the patient withdrawal criteria (Section 8.6); or
- Terminating the trial.

# 6. TRIAL OBJECTIVES AND ENDPOINTS

## 6.1. Core Period

### 6.1.1. Primary Objective

The primary objective of the study is to:

- Evaluate the safety and tolerability of up to 24 weeks of AG-348 administration in patients with PK deficiency.

### 6.1.2. Secondary Objectives

The secondary objectives of the study are to:

- Evaluate the PK of AG-348 and the metabolite AGI-8702.
- Evaluate the PD response of ATP and 2,3-DPG after administration of AG-348.
- Evaluate indicators of clinical activity of AG-348 in patients with PK deficiency, including changes in Hb, HCT, reticulocyte count, haptoglobin (Hp), carboxyhemoglobin (COHb), end tidal carbon monoxide (ETCO), lactate dehydrogenase (LDH), total and indirect bilirubin, erythropoietin (EPO), hepcidin, ferritin, and transferrin saturation (serum iron/iron binding capacity).

## 6.2. Extension Period

### 6.2.1. Primary Objective

The primary objective of the study is to:

- Evaluate the safety and tolerability of up to 30 months of AG-348 administration in patients with PK deficiency.

### 6.2.2. Secondary Objectives

The secondary objectives of the study are to:

- Evaluate the PK of AG-348 and the metabolite AGI-8702.
- Evaluate the PD response of ATP and 2,3-DPG after administration of AG-348.

- Evaluate indicators of clinical activity of AG-348 in patients with PK deficiency, including changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, hepcidin, ferritin, and transferrin saturation (serum iron/iron binding capacity).

## 6.3. Study Measures and Endpoints

### 6.3.1. Safety Measures and Endpoints

Safety will be evaluated by:

- Monitoring of AEs, including determination of SAEs and AEs leading to discontinuation; safety laboratory parameters (hematology, chemistry, urinalysis, coagulation); physical examination findings; vital signs (VS); 12 lead electrocardiograms (ECGs); and DXA scans. Adverse events will be graded using CTCAE, Version 4.03. Serum sex hormone levels (testosterone [total and free], estrone, and estradiol), bone turnover markers (serum osteocalcin-N-mid and serum C-terminal telopeptide [CTX]), 25-hydroxy vitamin D2 and D3, total cholesterol, high-density lipoprotein-cholesterol (HDL-C), and triglycerides will be monitored for evidence of potential inhibition of aromatase by AG-348. Menstruating female patients will also keep a paper-based menstrual cycle diary throughout the Core and Extension Periods.

### 6.3.2. Clinical Activity Measures and Endpoints

- Monitoring of potential indicators of clinical activity will include evaluating changes in Hb, HCT, reticulocyte count, Hp, COHb, ETCO (Core Period only), LDH, total and indirect bilirubin, EPO, hepcidin, ferritin, and transferrin saturation.

### 6.3.3. Pharmacokinetic and Pharmacodynamic Measures and Endpoints

The PK and PD profile of AG-348 will be evaluated by:

- Approximately the first 10 patients treated during the Core Period, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 15.1, Table 7. The remainder of treated patients will undergo limited PK sampling as detailed in Appendix 15.1, Table 8. During the Core Period, serial blood sampling for determination of concentration-time profiles of AG-348 and its metabolite AGI-8702 will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of AG-348 and AGI-8702 will be obtained. During the Extension Period, predose PK samples will be drawn for the measurement

of trough levels of AG-348 and AGI-8702 at each study visit (every 3 months; Appendix 15.1, Table 6). AG-348 and AGI-8702 will be analyzed using qualified assays to determine concentrations in plasma. Pharmacokinetic parameters on Day 1 and Day 15 will be computed using standard non-compartmental methods based on observed plasma AG-348 and AGI-8702 concentrations.

- Pharmacodynamic assessments during the Core Period will include 2,3-DPG, ATP (secondary objectives), [redacted] Approximately the first 10 patients treated during the Core Period will undergo extensive PD sampling as detailed in Appendix 15.1, Table 7. The remainder of treated patients will undergo limited PD sampling as detailed in Appendix 15.1, Table 8. During the Core Period, serial blood sampling for determination of levels of ATP and, 2,3-DPG will be conducted following the first dose and the morning Day15 dose, and additional trough levels of ATP and 2,3-DPG will be obtained. During the Extension Period, predose PD samples will be drawn for the measurement of trough levels of 2,3-DPG, ATP, [redacted] at each study visit (every 3 months; Appendix 15.1, Table 6). Adenosine triphosphate and 2,3-DPG will be analyzed using qualified assays to determine concentrations in whole blood. PD parameters on Day 1 and Day 15 will be computed based on observed whole blood ATP and 2,3-DPG concentrations.

[redacted]

# 7. INVESTIGATIONAL PLAN

## 7.1. Overall Study Design

Study AG348-C-003 is a Phase 2, open-label, two-arm, multicenter, randomized, dose-ranging study in adult patients with PK deficiency; the study will be divided in to a Core Period and an Extension Period. During the Core Period, patients will receive multiple doses of AG-348 for up to 24 weeks; patients who are eligible can enter the Extension Period to receive AG-348 for up to 2 years following the end of the Core Period. Patients with PK deficiency confirmed by RBC PK enzymatic assay performed at Screening will be eligible to participate in this study. At Week 25, patients who safely tolerate AG-348 and demonstrate clinical activity of AG-348 may be eligible to immediately roll over to the Extension Period for continued treatment. Patients who complete treatment at the end of the Core Period (24 weeks) will undergo follow-up assessment 4 weeks after the last dose of study drug. If a patient discontinues at any other time (including early discontinuation or discontinuation during the Core or Extension Period), follow-up assessments will be conducted 4 weeks after discontinuation. Patients with toxicity suspected to be related to study drug will continue follow-up until the AE resolves, is declared chronic by the Investigator, or the patient is lost to follow-up.

For the Core Period, up to 25 patients will be initially randomized on an open-label, 1:1 basis to each of two BID doses of AG-348 (up to 50 patients; see Figure 3, Study Schema). The dose of Arm 1 is 300 mg of AG-348 administered orally q12h (BID). The dose of Arm 2 is 50 mg of AG-348 administered orally q12h (BID). Randomization will be stratified by *PKR* mutation in order to maintain balance as much as possible across the dose arms for the specific mutations expected to be most frequently enrolled. The PKR mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H) and all other mutations ("other"). Mutation status is defined by the presence of at least one of the indicated mutations; patients with more than one stratified mutation will be assigned based on Sponsor's discretion.

The doses for each arm of the Core Period have been selected from the AG348-C-001 SAD study and AG348-C-002 MAD studies in healthy adult volunteers to represent the range of doses/exposures that were safely tolerated and resulted in maximal or near maximal PD effects on 2,3-DPG and ATP.

Because PK deficiency is a rare disease with a limited eligible patient population and because the underlying pathophysiology and clinical phenotype of affected patients is heterogeneous due to the wide variety of mutations in PKR that cause the disease, it is deemed important to focus closely on dose findings in this first-in-patient study. Therefore, in addition to initiating this study with 2 different doses of AG-348 administered q12h, a DRT will be established to review study data on a frequent basis and adapt the study design, dose and schedule of AG-348 as indicated.

The DRT will monitor safety on an on-going basis and meet at regular intervals of approximately every 6 weeks, or *ad hoc* as necessary, for as long as any patients are still in the Core Period to review AEs, VS, clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and ECGs. The DRT will also review available PK/PD data and indicators of clinical activity (e.g., changes from baseline in Hb). These DRT meetings will also include data review

for all patients that may be under treatment in the Extension Period. If there are no patients still being treated in the Core Period, and the only patients on treatment are those in the Extension Period, then the frequency of the DRT meetings will reduce to approximately every 3 months in order to match the frequency of patient visits (and new data collection) in the Extension Period. The DRT will be comprised of the study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer.

Beginning 6 weeks after the first patient is dosed in the Core Period or *ad hoc* as necessary, and proceeding according the schedule indicated above (approximately every 6 weeks during the Core Period, approximately every 3 months during the Extension Period once all patients have completed the Core Period), the DRT will review cumulative safety data, available PK/PD data, and clinical activity data. Based on the DRT's recurring, the DRT may exercise one or more of the following options during the Core Period:

- Continue treatment and enrollment in existing arms without change.
- Add 1 new dose arm (Arm 3) to enroll up to 25 patients at a dose to be determined; the dose for Arm 3 may be lower or higher than Arm 1 and Arm 2 doses, but will not exceed 360 mg q12h; and the dose regimen may be less frequent than q12h.
- Terminate or suspend enrollment to allow further review of clinical data in Arm 1 and/or Arm 2 (and/or potential Arm 3). Enrollment in an arm could be terminated or suspended to allow further review, for example, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity.
- Re-assign patient's doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the patients in the terminated arm will remain in their original arm, i.e., they will not count towards the enrollment quota of the arm whose dose and schedule is being adopted.
- Implement specific genotype restrictions to enrollment in one or more arms to ensure representation of patients with genotypes of greatest clinical relevance.

The DRT may exercise one or more of the following options during the Extension Period:

- Continue treatment without change;
- Re-assign patients' doses and schedule to an existing dose and schedule that has been determined to be safer, and/or have a better PD response, or produce signs of clinical activity;
- Terminate or suspend treatment to allow further review of clinical data (eg, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity).

The data that the DRT will review to make these decisions is expected to include, but are not necessarily limited to, the following:

- *Safety Observations:* all AEs; VS, clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and ECGs;
- *PK and PD Observations:* including changes in 2,3-DPG and ATP;

- *Indicators of Clinical Activity:* including changes in Hb, HCT, reticulocyte count, Hp, COHb, ETCO (Core Period only), LDH, EPO, hepcidin, total and indirect bilirubin, ferritin, and transferrin saturation.

If a third dose arm is implemented, the dose of AG-348 selected will not exceed 360 mg BID, as this was the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy volunteers. The PK/PD sampling schedule in a potential third dosing arm will be determined by Sponsor's discretion and may follow either the extensive or limited PK/PD sampling schedules as specified.

The DRT will monitor the safety data in an ongoing manner as described and may make a decision to terminate enrollment in an arm if unacceptable AEs are observed, PD activity is limited, and/or markers of clinical activity indicate no effect. Unacceptable AEs are defined as ≥ Grade 3 AEs (using National Cancer Institute (NCI) CTCAEv4.03) that are assessed as at least possibly related to AG-348, with the exception of Grade 3 headache, hot flash/flushing, nausea, vomiting, and/or diarrhea that is transient in duration (< 24 hours) or able to be medically managed to ≤ Grade 2 within 24 hours. The DRT may also exercise discretion to terminate enrollment in an arm if it observes a frequency of Grade 2 AEs that would make it difficult to support long-term dosing.

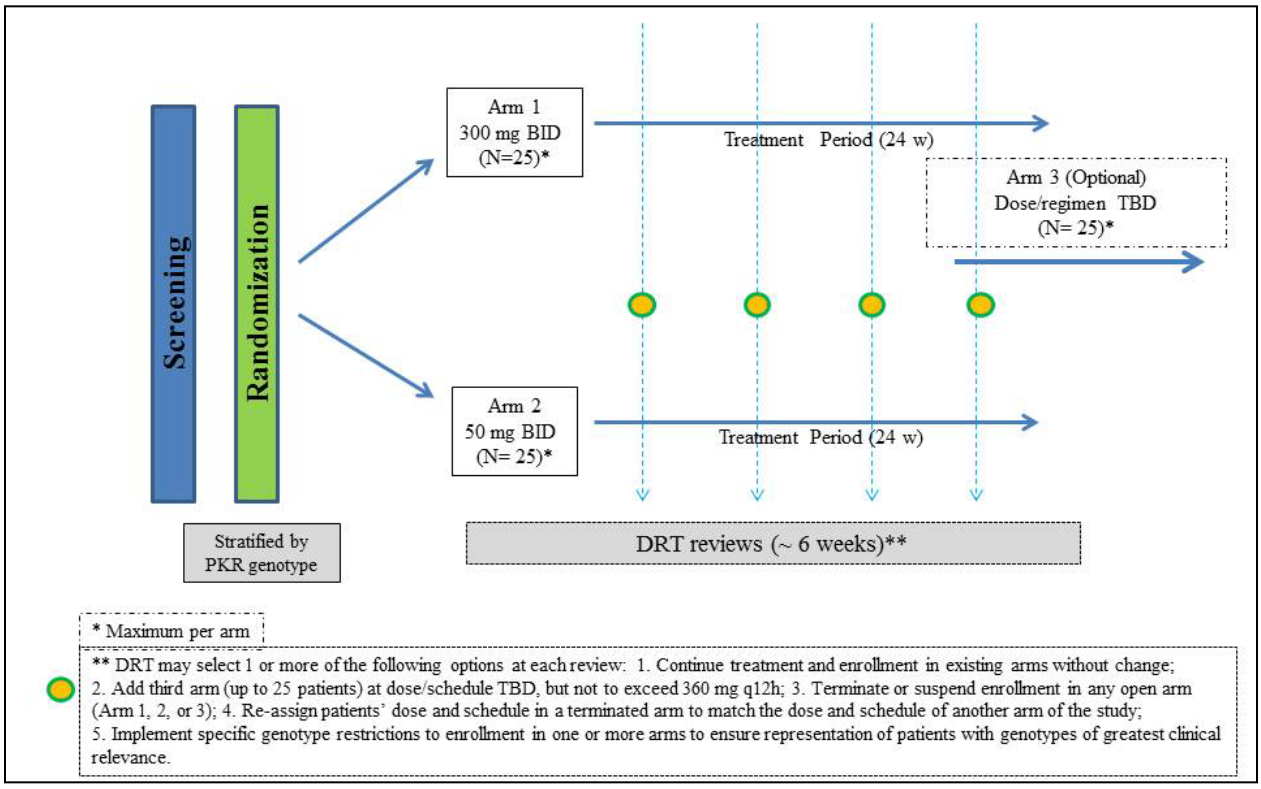
Patients in the Extension Period will continue on the dose they were randomized to in the Core Period, unless the DRT had reason to establish a different dose/schedule during the course of the Core Period (the DRT will not propose a dose higher than 360 mg q12h), or unless the treating Investigator exercises the option for intra-patient dose escalation (see Section 9.7.2).

Due to the potential for AG-348-mediated aromatase inhibition, DXA scan (hip and spine) will be performed at Screening (if patient has not had prior DXA scan within 3 months of Day 1) to obtain T and Z scores. These data are intended to serve as a baseline measure of bone mineral density for all enrolling patients, and are deemed of particular importance for those who may enter the longer term Extension Period after completing 24 weeks of treatment (Core Period). All patients will have a second DXA scan in the interval between Weeks 24 and 28 for the Core Period. Patients in the Extension Period will have additional DXA scans at Months 18 and 30.

As the number of enrolling arms changes in the study (for example, from 2 to 3), the randomization scheme will adjust to enable balanced randomization into each actively accruing arm. Randomization and stratification will cease in the event that only a single arm is left enrolling.

Depending on possible early termination of 1 or both of the initial 2 arms, or the addition of a third arm, the study could enroll up to a maximum of 75 patients.

**Figure 3: Study Schema: Core Period**




Abbreviations: BID (q12h) = twice-daily (every 12 hours); DRT = data review team; PKR = pyruvate kinase red blood cell isoform; TBD = to be determined; w = weeks.

The Investigator will monitor all patients for safety and tolerability. Modification of an individual patient's dose of AG-348 will be based on AEs and observed changes in Hb levels as detailed in Section 9.7.1 and Section 9.7.2.

Screening assessments will occur within 42 days prior to the first dose of study treatment. During the Core Treatment period, patients will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12), and monthly through Week 24 (Weeks 16, 20, and 24). Patients who safely tolerate AG 348 through Week 24 (Core Period) may be eligible to immediately enter the Extension Period for continued treatment. Study visits for safety and clinical activity assessments will occur approximately every 3 months during the Extension Period for up to 2 years after the end of the Core Period. All patients will undergo a follow-up assessment 4 weeks after the last dose of AG-348, regardless of whether this was due to early discontinuation, the last dose in the Core Period for a patient who chooses not to continue in the Extension Period, or the last dose of the Extension Period.

Safety assessments will include monitoring of AEs, including determination of SAEs and AEs leading to discontinuation; safety laboratory parameters (e.g., hematology, serum chemistry, coagulation studies, and urinalysis); physical examination findings; VS; 12 lead ECGs, and DXA scans. Additional safety assessments will include monitoring of sex hormone levels (testosterone [total and free], estrone, and estradiol), bone turnover markers (osteocalcin-N-mid and CTX), 25-hydroxy vitamin D2 and D3 levels, total cholesterol, HDL-C, and triglycerides. Follow-up

assessments will be conducted on Day 197 (Week 28) for patients who do not enter the Extension Period and will include physical examination, weight, performance status, VS, 12-lead ECGs, laboratory evaluations (hematology, Hp, EPO levels, serum chemistry, coagulation studies, urinalysis; lipids, hormonal testing), AEs, and transfusion record. These follow-up assessments will be performed approximately 4 weeks after discontinuation of AG-348 for patients who discontinue prior to completion of the Core Period and for those who discontinue in the Extension Period. Menstruating female patients will also be required to keep a paper-based menstrual cycle diary throughout the study.

Pharmacokinetic assessments will include serial blood sampling for PK profiles of AG-348 and its metabolite AGI-8702. Pharmacodynamic evaluations will include serial blood sampling for determination of levels of ATP and 2,3 DPG. Extensive PK/PD sampling will be conducted on the first approximately 10 patients total treated in Arms 1 and 2 of the Core Period (see Appendix 15.1, Table 7) while limited PK/PD sampling will be conducted on the remainder of treated patients (see Appendix 15.1, Table 8). Limited trough sampling will be conducted every 3 months during the Extension Period (Appendix 15.1, Table 6).

## 7.2. Justification of the Study Design

The primary and secondary objectives of this study are to evaluate the safety, tolerability, PK and PD, and indicators of clinical activity of AG-348 in patients with PK deficiency. The choice of dose and schedule of administration of AG -348 for Arms 1 and 2 was based on the highest safely tolerated dose (Arm 1: 300 mg BID) and the lowest dose with potentially relevant PD activity (Arm 2: 50 mg BID) from the forerunner AG348-C-002 MAD study in healthy volunteers. Decisions regarding continuing enrollment and treatment in these initial dose arms and/or implementation of an additional dose arm will be based on DRT review of safety, PK and PD data, and indicators of clinical activity collected from all patients treated in Arm 1 and Arm 2. This design was chosen to minimize risk to patients while allowing evaluation of safe and pharmacologically active dose levels of AG-348, and to allow the necessary flexibility to adjust dose and schedule should the safety, tolerability, PK, and/or PD be different in patients with PK deficiency compared with healthy volunteers.

Additional safety measures intended to minimize risk to patients include monitoring of AEs by the DRT and specified provisions for individual patient dose modification as needed for safety and (potentially) large increases in Hb level (Section 9.7.1 and Section 9.7.2). Measures intended to maximize the opportunity for patients with demonstrated safety and tolerability to continue to derive benefit from any observed clinical activity of AG-348 include the option for continued treatment in the Extension Period.

A comprehensive series of safety evaluations, including laboratory parameters, physical examinations, VS, 12-lead ECGs, and monitoring for SAEs and AEs, will be conducted to evaluate the safety profile of AG-348 and to aid in the determination of the recommended dose for continued development.

The study includes serial blood sampling across at least two different doses of the study drug to assess its PK and PD profiles.

Consistent with the design of many Phase 2 studies, preliminary evaluation of the potential PD and clinical activity of AG-348 are secondary objectives of this study. The latter will include assessments of the PK/PD relationship between AG-348 and the biomarkers ATP and 2,3-DPG, [redacted]

## 7.3. Rationale for the Starting Dose, Dose Range, and Duration of Dosing

Prior to execution of this study, Agios conducted two clinical studies of AG-348 in healthy volunteers, including a SAD study (AG348-C-001) and a MAD (14 day q12h) study (AG348-C-002). Available details of these studies are discussed in the current Investigator's Brochure (IB). Between these two studies, 72 healthy human subjects have been dosed with AG-348. *In vitro* investigations, also reported in the IB, had previously demonstrated that AG-348 increased the activity of wild-type PKR approximately to the same extent as it did a series a recombinant mPKRs. Therefore it was deemed reasonable to study the safety, tolerability, PK, and PD of AG-348 in healthy subjects in a controlled Phase 1 setting as a more efficient means of obtaining information than in the actual rare disease population of patients with PK deficiency.

The MAD study demonstrated that the exposures produced by AG-348 doses from 60 mg q12h to 360 mg q12h (including 120 mg q24h) resulted in maximal changes from baseline for the PD markers 2,3-DPG (reduction from baseline) and ATP (increase from baseline). The exposures resulting from doses less than 60 mg q12h were of lesser magnitude and the exposures resulting from doses greater than 360 mg q12h were of no greater magnitude than the aforementioned range. Therefore the starting doses for this first dose ranging study in patients with PK deficiency were selected to be 300 mg q12h (Arm 1) and 50 mg q12h (Arm 2). These doses were demonstrated to be safe and tolerable in the healthy volunteer studies. The availability of ATP is proposed as being critical for optimally maintaining RBC membrane integrity (see Section 5.1). The dose ranges from 50 mg q12h to 300 mg q12h may result in clinically effective modulation of PKR in PK deficiency patients if the mutated enzyme is responsive to AG-348 in a similar manner to the wild-type enzyme in healthy subjects. However, there are many different mutations in PKR that result in PK deficiency, and these mutations produce variable effects on the enzyme in terms of catalytic activity and thermal stability. It is not known if different mutations will respond clinically in a similar manner to the same exposure to AG-348. Therefore, it is prudent to study the range of safe and pharmacodynamically relevant doses as specified in this study, and to allow flexibility for the DRT to analyze the evolving study data to adapt the dose and schedule of administration of AG-348 to produce the optimal combination of safety, tolerability, and PD, and, potentially, clinical response.

**Justification for Duration of Dosing**

The initial treatment duration of 24 weeks (6 months) for the Core Period was chosen for this proof-of-concept trial for 2 principal reasons: 1) to begin establishing a safety database addressing the chronic administration of AG-348; and 2) to allow sufficient time for clinical response to treatment to appear.

It is anticipated that this treatment, if successful, may be taken for life, as PK deficiency is a genetically determined inborn error of metabolism. Therefore, it is important to begin to investigate the long-term safety of the treatment. As will be discussed below, the safety package supports the treatment duration of 6 months.

Red blood cell turnover is typically 120 days (4 months), although it may be shorter in some populations of pyruvate kinase deficient RBCs (Mentzer, et al. 1971). Different PKR mutations produce a variety of physiologic consequences. In mutations where normal or nearly normal levels of PKR protein persist, but the protein's catalytic function is impaired, an improvement in PKR functional activity might be seen relatively quickly upon exposure to AG-348. However, in cases where the PKR mutation results in an unstable mutant, PKR protein levels may be low and additional time may be required for stabilization of the newly synthesized mutant enzyme in developing bone marrow erythroblasts so that eventually more mature RBCs may be produced with more nearly normal levels of functional PKR protein.

This study plans up to 24 weeks of dosing with AG-348 during the Core Period with the possibility of continued dosing beyond 24 weeks during the Extension Period in patients for whom AG-348 is safely tolerated and demonstrates clinical activity. The International Conference on Harmonisation (ICH) Guideline M3(R2) on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals, recommends that clinical trials of a duration between 2 weeks and 6 months should be supported by repeat-dose toxicity studies of at least equivalent duration, and clinical trials lasting beyond 6 months should be supported by 6-month rodent and 9-month non-rodent studies. This guidance allows for clinical trials of longer duration to be initiated based on 3 months of nonclinical data, and for clinical dosing in serious or life-threatening indications to be extended based on complete chronic rodent data and in-life and necropsy data in the non-rodent (and complete histopathology in the non-rodent provided within an additional 3 months).

For the current investigational product (AG-348), 13-week, repeat dose toxicology studies in the rat and monkey have been completed and are summarized in Section 5.2.1.4 of this protocol and in the current Investigator Brochure prepared to support initiation of this clinical study. Considering that PK deficiency is a serious condition with an unmet medical need, it is appropriate to initiate this clinical study with the available nonclinical data. Six-month rodent and nine-month monkey toxicology studies were initiated in January 2015, and the Sponsor will report the results of these studies in each applicable regulatory region as required before any patients will be treated for greater than 6 months (i.e., enter the Extension Period).

## 7.4. Criteria for Study Termination

This study may be prematurely terminated if, in the opinion of the Sponsor, there is sufficient reasonable cause. In the event of such action, written notification documenting the reason for study termination will be provided to each Investigator.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to patients;
- Insufficient adherence to protocol requirements;
- Plans to modify, suspend, or discontinue the development of the study drug;

- Other administrative reasons.

Should the study be closed prematurely, all study materials must be returned to the Sponsor or the Sponsor's designee. Patients who have participated in the Extension Period may be able to screen for eligibility in a study for a second generation allosteric PKR activator, if such a compound is available at that time, and after a suitable washout period has been observed.

# 8. STUDY POPULATION

## 8.1. Number of Patients

Up to approximately 75 patients may be enrolled in this study.

## 8.2. Inclusion Criteria

For entry into the Core Period, patients must meet all of the following criteria during the Screening or other specified period:

1. Signed written informed consent obtained prior to performing any study procedure, including screening procedures.
2. Male or female, aged 18 years and older.
3. Known medical history of PK deficiency.
4. All patients must have documented clinical laboratory confirmation of PK deficiency by RBC pyruvate kinase enzymatic assay performed at Screening either by a designated central laboratory or by any participating investigative site's local hematology laboratory. Patients with prior documentation of PK deficiency by RBC enzymatic assay will have a reconfirmation of this result during Screening as a condition of enrollment.
    a. In the event that a patient's screening pyruvate kinase enzymatic assay is negative (i.e., shows normal pyruvate kinase activity), the patient will be eligible for enrollment if the genotyping shows a mutant genotype that has been previously documented in the literature to be associated with PK deficiency. If the genotyping shows a previously undescribed mutation in the PKR gene, then the eligibility for enrollment will be determined on a case-by-case basis by the Coordinating Investigator and Medical Monitor in discussion with the Investigator. If no mutation is defined, then the patient will not be eligible.
5. ALL patients must have a blood sample for genotypic characterization of the mutant PKR gene performed by the designated central laboratory at Screening. The designated central laboratory-determined genotype will generally serve as the basis for genotyping for enrollment. However, patients whose genotype has already been determined by another laboratory may be enrolled on the basis of that report, with the approval of the Medical Monitor, in case of unexpected delay in return of the designated central laboratory result during the Screening Period. Enrollment on the basis of a result from a laboratory other than the designated central genotyping laboratory does not relieve the inclusion requirement that ALL patients must have a sample sent to the designated central genotyping laboratory.
6. All patients must have genotypic characterization of the UGT1A1 gene performed by a designated central laboratory to document whether they may have underlying Gilbert's Disease. Patients with Gilbert's Disease are eligible to enroll.
7. Males must have Hb $\leq$ 12.0 g/dL; females must have Hb $\leq$ 11.0 g/dL.

8. All patients must be considered transfusion independent as defined by: no greater than 3 units of RBCs transfused in the 12-month period up to the first day of study dosing and no transfusions within 4 months of first day of study dosing. Patients who have received more transfusion support than described above will be evaluated for eligibility on a case-by-case basis by the Medical Monitor.
9. Eligible patients may still have their spleens in place, or may have undergone prior splenectomy. For splenectomized patients:
    a. Must have undergone their procedure at least 6 months prior to Screening.
    b. Must be current in their vaccinations for Pneumococcal Conjugate (PCV13), Pneumococcal Polysaccharide (PPSV23), Quadrivalent Meningococcal vaccine, and *Haemophilus influenzae* type b as recommended by Centers for Disease Control and Prevention's (CDC) Advisory Committee on Immunization Practices (ACIP) or immunization advisory groups in Canada and the European Union (for patients enrolled in Canada and the EU). [http://www.cdc.gov/vaccines/schedules/downloads/adult/adult-schedule.pdf] [Any missing vaccinations may be administered starting with the Screening Period and during the trial following the initiation of AG-348 dosing as necessary according to recommended vaccination guidance.]
10. Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 2 (Appendix 15.2).
11. Patients must be taking at least 1 mg of folic acid daily for at least 21 days prior to first dose and continued daily during study participation.
12. Adequate organ function, defined as:
    a. Serum AST ≤ 2.5 × ULN (unless the increased AST is assessed by the Investigator as due to hemolysis and/or hepatic iron deposition); and ALT ≤ 2.5 × ULN (unless the increased ALT is assessed by the Investigator as due to hepatic iron deposition).
    b. Normal or elevated levels of serum bilirubin. In patients with serum bilirubin > ULN, the elevation must be attributed to hemolysis with or without Gilbert's syndrome and must not be choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease.
    c. Serum creatinine ≤ 1.25 × ULN. If serum creatinine > 1.25 × ULN, then 24-hour measured or calculated (Cockcroft-Gault) glomerular filtration rate (GFR) ≥ 60 mL/min.
    d. Absolute neutrophil count (ANC) ≥ 1.0 × 109/L.
    e. Platelet count ≥ 100 × 109/L.
    f. Activated partial thromboplastin time (aPTT) and international normalized ratio (INR) ≤ 1.25 × ULN, unless the patient is receiving therapeutic anticoagulants.
13. Women of childbearing potential (WOCBP) must agree to abstain from sexual intercourse or to use an acceptable/effective method of contraception (i.e., condom plus spermicide, condom plus oral contraceptive, condom plus intrauterine device [IUD], condom plus diaphragm with spermicide) from as soon as feasible during the Screening

period until 30 days following the last dose of AG-348. Abstinence is an acceptable method only when this is in line with the normal life style of the patient, meaning that the patient plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence, e.g. calendar, sympathothermal and post-ovulation methods, and withdrawal are not acceptable methods of contraception.

a. WOCBP include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, and/or bilateral oophorectomy) or is not post-menopausal. Post-menopausal is defined as:

i. Amenorrhea ≥ 12 consecutive months without another cause, and a documented serum follicle stimulating hormone (FSH) level > 35 mIU/mL;

ii. Amenorrhea≥ 12 consecutive months in women ≥ 62 years old (FSH testing is not required).

14. WOCBP must have a negative serum or urine pregnancy test within 72 hours before start of AG-348 dosing.
15. Women must not be breastfeeding.
16. Male patients, with the exception of those who have undergone vasectomy at least 6 months prior to Screening, must agree to abstain from sexual intercourse or, if sexually active, to use a condom with spermicide as contraception (regardless of their female partner's childbearing potential or their partner's use of their own contraception) from Day 1 of dosing until 30 days following the last dose of AG-348. Abstinence is an acceptable method only when this is in line with the normal life style of the patient, meaning that the patient plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence, e.g. selective timing of intercourse based on partner's calendar, sympathothermal and post-ovulation methods, and withdrawal are not acceptable methods of contraception.

For entry into the Extension Period, patients must meet the following criteria:

17. Signed written informed consent obtained prior to performing any study procedure during the Extension Period.
18. Patient must have completed 24 weeks of treatment during the Core Study and tolerated AG-348 (defined as having completed 24 weeks with or without permitted dose modifications).
19. The patient’s treating Investigator agrees that there is a potential for clinical benefit to continued treatment and recommends participation in the Extension Period
20. The Sponsor's designated Medical Monitor or Responsible Medical Officer approves the patient’s participation in the Extension Period
21. As applicable, the patient must agree to continue to follow the same sexual abstinence/contraception rules as stated in Inclusion Criteria 13 and 16.

## 8.3. Exclusion Criteria

Patients who meet any of the following criteria at Screening or prior to dosing on Day 1 will not be enrolled in Core Period:

1. Hemoglobin level > 12.0 g/dL if male; Hb > 11.0 g/dL if female.
2. Additional diagnosis of any other congenital or acquired blood disorder, including glucose-6-phosphate-dehydrogenase (G6PD) deficiency, or any other hemolytic anemia process except for mild allo-immunization as a consequence of transfusion therapy.
3. Iron overload (hemosiderosis or concurrent hemochromatosis) sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac, hepatic, or pancreatic insufficiency.
4. Prior bone marrow or stem cell transplant.
5. Clinically symptomatic cholelithiasis or cholecystitis. (Prior cholecystectomy is not exclusionary. Patients with symptomatic cholelithiasis or cholecystitis may be rescreened once the disorder has been treated and clinical symptoms have resolved.)
6. Currently enrolled in another therapeutic clinical trial involving on-going therapy with any investigational or marketed product or placebo. Concurrent participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480) is permitted.
7. Exposure to any investigational drug, device, or procedure within 28 days prior to Screening or during trial participation.
8. Concurrent medical condition that could compromise participation in the study such as:
    a. Poorly controlled hypertension (defined as systolic blood pressure (BP) > 150 mm Hg or diastolic BP > 90 mm Hg) refractory to medical management.
    b. History of recent (within < 6 months from Screening date) congestive heart failure; myocardial infarction or unstable angina pectoris; or hemorrhagic, embolic, or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.
    c. Currently active infection requiring the use of parenteral anti-microbial agents or that is ≥ Grade 3 (CTCAEv4.03) within 6 months of first dose.
    d. A pattern or frequency of post-splenectomy sepsis that in the assessment of the Investigator could reasonably be expected to interfere with the ability of the patient to complete the 24 week Core Period study participation.
    e. Positive test for hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV) antibody with signs of active Hepatitis B or C virus infection.
    f. Positive test for human immunodeficiency virus (HIV) 1 or 2 antibody.
    g. Diabetes mellitus judged to be in poor control by the Investigator or requiring > 3 anti-diabetic agents counting insulin; use of insulin *per se* is not exclusionary.
    h. History of any primary malignancy with the exception of: curatively treated non-melanomatous skin cancer; curatively treated cervical or breast carcinoma *in situ*;

or other primary tumor treated with curative intent and no known active disease present and no treatment administered during the last 3 years.

9. Undergone major surgery within 6 months of first dose.
10. Current or recent history of psychiatric disorder that in the opinion of the Investigator or Medical Monitor could compromise the ability of the patient to cooperate with study visits and procedures.
11. Use of any of the restricted list of products known to strongly inhibit CYP3A4 metabolism (Appendix 15.4, Table 9) within 5 days prior to Day 1 dosing; or to strongly induce CYP3A4 metabolism (Appendix 15.4, Table 10) within 28 days prior to Day 1 dosing; or to strongly inhibit P-gp transporter (Appendix 15.4, Table 11) within 5 days prior to Day 1 dosing; or digoxin within 5 days prior to Day 1 dosing.
12. Serum bilirubin > ULN attributable to factors other than hemolysis and/or Gilbert's syndrome.
13. Male patients with heart-rate corrected QT interval -Fridericia's method (QTcF) interval > 450 msec, or female patients with QTcF interval > 470 msec with the exception of patients with a left bundle branch block (LBBB). Medical Monitor approval needed in patients with a LBBB.
14. Cardiac dysrhythmias judged as clinically significant by the Investigator or requiring therapy with drugs that are primarily substrates of CYP3A4.
15. History of allergy to sulfonamides if characterized by acute hemolytic anemia, anaphylaxis, or rash of erythema multiforme type or Stevens-Johnson syndrome.
16. Any other medical or psychological condition deemed by the Investigator to be likely to interfere with a patient’s ability to understand and sign informed consent; cooperate with study visits, tests, and procedures; or otherwise safely and reliably participate in the study.

Patients will not be allowed to enter the Extension Period if:

17. The patient experienced AEs during the Core Period that are considered by the treating Investigator or the Sponsor’s designated Medical Monitor or Responsible Medical Officer to pose a significant safety risk to the patient if treatment were to be extended.

## 8.4. Patient Identification and Registration

Patients who are candidates for enrollment into the study will be evaluated for eligibility by the Investigator to ensure that the inclusion and exclusion criteria (see Section 8.2 and Section 8.3, respectively) have been satisfied and that the patient is eligible for participation in this clinical study. The site will submit to the Sponsor an Eligibility form for each eligible patient and the Medical Monitor will confirm eligibility for all patients prior to receipt of the first dose of AG-348.

## 8.5. Patient Randomization

Patients who have been confirmed as eligible will be randomized in an equal ratio to a treatment arm (e.g., 1:1 or 1:1:1 depending on which arms are open). The site will provide a request for

randomization form (including the patient's confirmed genotype) to the study Medical Monitor. The randomization will be stratified by PKR mutation in order to maintain balance across the dose arms for the specific mutations expected to be most frequently enrolled. The PKR mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H) and all other mutations ("other"). Since this is an open label study, randomization will not be blinded.

Please refer to the study manual for the randomization procedure.

## 8.6. Patient Withdrawal Criteria

Patients have the right to withdraw from the study at any time for any reason. Patients will be withdrawn from study-related procedures and treatments under the following conditions:

- Withdrawal of consent;
- Experiences unacceptable toxicity;
- Development of an intercurrent medical condition that precludes further participation in the trial;
- Patient requires use of a prohibited concomitant medication (Section 9.11.2);
- Investigator decision;
- Protocol violation: non-adherence to protocol requirements;
- Pregnancy;
- Lost to follow-up.

Should a patient decide to withdraw, all efforts will be made to complete and report the protocol-defined study observations up to the time of the patient's withdrawal as completely as possible and to determine the reason for withdrawal.

In the event a patient is withdrawn from the study, the Medical Monitor must be informed. If there is a medical reason for withdrawal, the patient will remain under the supervision of the Investigator until satisfactory health is returned.

When a patient withdraws from the study, the primary reason for discontinuation must be recorded in the appropriate section of the electronic case report form (eCRF) and all efforts will be made to complete and report final study observations as thoroughly as possible.

All AEs should be followed until resolution or for a period of 30 days from the last dose of study drug, whichever is shorter. Patients with toxicity suspected to be related to study drug will continue follow-up until the AE resolves, is declared chronic by the Investigator, or the patient is lost to follow-up.

## 8.7. Replacement of Patients

Patients who drop out of the Core Period prior to completing the first 12 weeks of assigned dosing for reasons other than AEs may be replaced at the Sponsor's discretion.

# 9. STUDY TREATMENT

## 9.1. Description of Study Drug

AG-348 sulfate hydrate capsules will be provided as 5 mg, 25 mg, or 100 mg (free-base equivalent) of AG-348 sulfate hydrate without excipients in dark green opaque (5 mg), Swedish orange (25 mg), or white opaque (100 mg) gelatin capsules (size 2 capsules for all dose strengths).

All study drugs are for investigational use only and are to be used only within the context of this study. All study drug products will be supplied by the Sponsor. The Sponsor reserves the right to discontinue the supply of any specific capsule strength should the evolving trial experience demonstrate that the specific capsule strength fills no additional need beyond the other capsule strengths available. Please see the Investigator's Brochure for further details regarding study drug.

## 9.2. Study Drug Packaging and Labeling

AG-348 sulfate hydrate capsules are packaged in white, high-density polyethylene (HDPE) induction sealed bottles with a child-resistant screw cap.

Packaging and labeling will be prepared to meet all regulatory requirements.

## 9.3. Study Drug Storage

AG-348 sulfate hydrate drug capsules must be stored at room temperature of 15 to 30°C (59 - 86°F).

All study drug products must be stored in a secure, limited-access location and may be dispensed only by the Investigator or by a member of the staff specifically authorized by the Investigator.

## 9.4. Method of Assigning Patients to Treatment

Up to a maximum of 25 patients will be randomized to any one of the dosing arms in this study. Randomization will be balanced 1:1 or 1:1:1, depending on whether 2 or potentially 3 arms are open, and will be stratified by PKR mutation (see Section 8.5). The dose and schedule of AG-348 each patient receives will be dependent upon which dose arm is open for enrollment when the patient qualifies for and is randomized into the study. Patients in the Extension Period will continue on the dose they were randomized to in the Core Period, unless the DRT had reason to establish a different dose/schedule during the course of the Core Period (the DRT will not propose a dose higher than 360 mg q12h).

## 9.5. Blinding

This is an open-label study; no blinding methods will be used.

## 9.6. Study Drug Preparation and Administration

For the initial two treatment arms, (Arm 1 and Arm 2) in the Core Period, AG-348 will be administered orally BID (approximately q12h with a minimum of 10 hours between doses) over a 24 week treatment period. Starting with Day 1, dosing will be continuous; there will be no rest periods. Patients who do not meet any of the treatment withdrawal criteria (see Section 8.5) may continue treatment for the entire 24-week treatment period.

Patients will be dispensed the appropriate number of Sponsor-packaged, labeled bottles to allow for 28 days of dosing until the next scheduled visit. The amount of study drug dispensed should be sufficient to provide an adequate reserve supply of AG-348 to ensure uninterrupted dosing in the event of an unexpected delay for the next scheduled study visit (7 extra days of dosing supply is recommended during the Core Period; 14 extra days is recommended during the Extension Period).

Patients will be given a dosing diary to be used for each 28-day dosing period. They should record relevant information regarding their study drug in the diary (e.g., confirmation that each daily dose was taken, reasons for missed doses).

Treatment compliance will be assessed based on return of unused drug and the dosing diary (see Section 9.9).

Patients should be instructed to take their daily dose at approximately the same times each day except for dosing on in-clinic visiting days.

Patients who undergo extensive PK/PD sampling during the Core Period (see Appendix 15.1, Table 7) should be instructed from Week 3 on to bring the AM dose with them for in-clinic visits and to ingest the dose following PK/PD blood draws.

Patients receiving limited PK/PD sampling during the Core Period (see Appendix 15.1, Table 8) should be instructed to bring the AM dose with them for all in-clinic visits and to take the AM dose following PK/PD blood draws.

Patients receiving extensive PK/PD sampling on Day 1 and 15 will also have limited PK/PD on other visit days. As a general rule, regardless of extensive or limited schedule, patients will bring in the AM dose for all visits and take this dose following PK/PD blood draws. Patients not continuing into the Extension Period are not required to take the Week 24/Day 169 morning dose of AG-348 after the required PK/PD blood samples are collected, as these patients will be discontinuing the study.

A minimum of 10 hours between the AM and PM dose will be required on those dosing days. Each dose should be taken with a glass of water and consumed over as short a time as possible. AG-348 may be taken with or without food. Patients should be instructed to swallow capsules whole and to not chew the capsules. For patients who have difficulty swallowing tablet(s), the Medical Monitor should be contacted to discuss administration.

Patients will receive their first dose of AG-348 in the clinic on Day 1 and then may take the remaining doses on an outpatient basis (except for in-clinic visit days, as described above).

## 9.7. Criteria for Dose Escalation, Dose Modification, or Discontinuation of Study Drug

Intra-patient dose escalations will be permitted in this study under 2 circumstances. First, the DRT may decide to re-assign patients' doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the patients in the terminated arm will remain in their originally assigned arm; i.e., they will not count towards the enrollment quota of the arm whose dose and schedule is being adopted. Second, a treating Investigator, with Medical Monitor approval, may elect to increase the dose for a patient in Arm 2 (50 mg BID) or a potential third arm of the study (if < 300 mg BID) to 300 mg BID if the patient is adequately tolerating his/her current dose and if their Hb has not reached at least the lower limit of the normal gender-adjusted reference range as specified by the designated central laboratory (male: 13.0 g/dL; female: 11.6 g/dL) after at least 12 weeks of treatment in the Core Period. The patient must have completed the 12-week visit during the Core Period and had all assigned tests/procedures for that visit before an intra-patient dose escalation will be allowed. An intra-patient dose escalation may also be made later than the 12-week visit in the Core Period or anytime during the Extension Period.

All dosing modifications, as outlined below, will be implemented following discussions with the Medical Monitor. The same criteria for dose modifications or discontinuation of study drug apply in the Extension Period as in the Core Period.

Patients should be advised not to discontinue dosing without first speaking with the treating Investigator—abrupt discontinuation of AG-348 dosing in a patient who experience a substantial increase in Hb may result in withdrawal hemolysis.

### 9.7.1. Dose Modification for Safety

The Investigator will monitor all patients for safety and tolerability. Modification of the patient's dose of AG-348 will be based on AEs and observed changes in Hb levels (see Section 9.7.2).

**Table 1: Dose Modification for Adverse Events Deemed at Least Possibly Related to AG-348**

| Adverse Events(s) | AG-348 Dose Adjustment |
|---|---|
| Grade 1 | None required. |
| Grade 2 | None required; Investigator and Medical Monitor judgment to manage as for Grade 3. |
| Grade 3 | Suspend dosing[1];<br>If event resolves to Grade 1 or baseline within approximately 14 days of suspension, resume dosing with at least a 1 dose level reduction and discuss the advisability of additional dose modification with the Medical Monitor (see Table 2 below).<br>If event does not resolve to Grade 1 or baseline within approximately 14 days of suspension, permanently discontinue dosing, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor. |
| Grade 4 | Permanently discontinue dosing, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor. |

[1] A related AE of Grade 3 severity would generally be expected to require suspension of dosing with AG-348. However, in some circumstances in which the Hb concentration has normalized prior to the onset of the related AE, the Investigator may elect to discuss with the Medical Monitor tapering the dose of AG-348 in order to mitigate potential withdrawal hemolysis (see Section 5.2.2.3 and Section 9.7.2).

Dose modifications for Grade 2 and 3 AEs should be maintained long enough for the Investigator to be confident that the reduced dose is being well tolerated before considering a request of the Medical Monitor to permit subsequent re-escalation to the former dose level. Dosing for an individual patient will be discontinued permanently for Grade 3 and Grade 4 AEs that do not resolve to Grade 1 or baseline within approximately 14 days of suspension of dosing, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor. No patients may be re-escalated to their former dose level after a dose modification without discussion with the Medical Monitor. If following the first dose reduction for a Grade 3 AE the patient experiences a second occurrence of the same Grade 3 AE, then treatment with AG-348 must be immediately and permanently discontinued.

It should be noted that if the initial dose of 300 mg BID selected for Arm 1 demonstrates an unacceptable safety profile resulting in multiple patients undergoing dose modifications, the DRT may exercise its option to re-assign these patients' dose and schedule to match the dose and schedule of another study arm (for example, Arm 2 of the study, or to match the dose and schedule of a [potential] Arm 3, if implemented).

### 9.7.2. Dose Modification for Increase in Hemoglobin Level

It is presently unknown to what magnitude, how rapidly, or even whether, AG-348 will result in increased Hb levels in patients with PK deficiency across the variety of potential PKR mutations that may be encountered during this study. Moreover, it is unknown whether any potential increases in Hb experienced by patients in this study may be a safety risk. As a conservative measure, this study incorporates guidance for dose modification based on potential large increases in Hb levels that exceed the midpoint of the typical normal range by gender. The intent

of this guidance is to strike a balance between abruptly stopping AG-348 treatment, thereby potentially risking withdrawal hemolysis, versus reducing the dose sufficiently to allow an opportunity for Hb to settle more gradually to a level less than the midpoint of the typical normal range by gender. The Investigator will monitor all patients for changes in Hb levels and should adjust the dose of AG-348 as outlined in Table 2 per the following guidelines and with Medical Monitor approval.

- Males: If Hb > 15.0 g/dL and confirmed with a second test, reduce dose by at least 1 dose level (Table 2) and discuss the advisability of additional dose modification or suspension with the Medical Monitor.
- Females: If Hb > 13.5 g/dL and confirmed with a second test, reduce dose by at least 1 dose level (Table 2) and discuss the advisability of additional dose modification or suspension with the Medical Monitor.
- The treating Investigator will discuss with the Medical Monitor questions relating to additional dose modifications and the need for additional unscheduled Hb monitoring on an as needed basis.

**Table 2: Dose Reduction Table (by Dosing Arm)**

| Dose Group | Starting Dose | 1st Dose Reduction | 2nd Dose Reduction |
|---|---|---|---|
| Arm 1 | 300 mg BID | 200 mg BID | 100 mg BID |
| Arm 2 | 50 mg BID | 25 mg BID | TBD[1] |
| Potential Arm 3 | TBD | To approximately 50-66% of initial dose | To approximately 25-33% of initial dose |

[1] Dose to be determined by Medical Monitor.

Hemoglobin levels above the ULN by gender should be reported as an AE, graded per the CTCAEv4.03, according to the guidance provided in Section 11.2.

### 9.7.3. Stopping Criteria

Dosing for an individual patient will be discontinued permanently for Grade 3 AEs that do not resolve to Grade 1 or baseline within approximately 14 days of suspension of dosing, and for Grade 4 AEs, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor (Section 9.7.1). Other reasons for treatment termination are provided in Section 8.5.

## 9.8. Duration of Patient Participation

The duration of treatment for all patients will be up to 24 weeks in the Core Period. Patients who safely tolerate AG-348 and for whom the Investigator agrees with continuation of AG-348 treatment may be eligible to immediately roll over to the Extension Period for continued treatment (up to 2 years following completion of the Core Period).

## 9.9. Treatment Compliance

During in-clinic visits, doses of AG-348 will be ingested by the patient under the supervision of clinical facility personnel. For at-home dosing, patients will be given a dosing diary to be used

for the duration of the 24-week Core Treatment Period; the diary will also be used by patients who roll over to the Extension Period. Patients should record relevant information regarding their study drug in the diary (e.g., confirmation that each daily dose was taken, reasons for missed doses) and return the diary at each study visit.

## 9.10. Study Drug Accountability

Accountability for the study drug at the clinical facility is the responsibility of the Investigator. The Investigator will ensure that the study drug is used only in accordance with this protocol. Where allowed, the Investigator may choose to assign drug accountability responsibilities to a pharmacist or other appropriate individual.

The Investigator or delegate will maintain accurate drug accountability records indicating the drug's delivery date to the site, inventory at the site, use by each patient, and return to Agios or its designee (or disposal of the drug, if approved by Agios). These records will adequately document that the patients were provided the doses as specified in the protocol and should reconcile all study drug received from Agios. Accountability records will include dates, quantities, batch/serial numbers, expiration dates (if applicable), and patient numbers. An unblinded monitor will review drug accountability at the site on a schedule agreed to by the Sponsor.

Study drug must not be used for any purpose other than the present study.

All unused and used study drug will be retained at the site until it is inventoried by the Study Monitor after database lock has occurred. All used, unused or expired study drug will be returned to Agios or its designee or, if authorized, disposed of at the study site per the site's Standard Operating Procedures (SOPs) and documented. All material containing AG-348 will be treated and disposed of as hazardous waste in accordance with governing regulations.

## 9.11. Prior and Concomitant Medications and Treatments

### 9.11.1. Prior Medications and Procedures

All medications administered and procedures conducted within 28 days prior to, or from Screening Visit #1 until the first day of study drug administration, whichever interval is longer, are to be recorded on the source documentation and included in the eCRF.

### 9.11.2. Prohibited Concomitant Therapy

All concomitant medications and procedures administered from 28 days prior to, or from Screening Visit #1 until the first day of study drug administration, whichever interval is longer, through the last Follow-up Visit must be recorded in the appropriate section of the source documentation and eCRF along with dosage information, dates of administration, and reason for use.

The following are prohibited at all times during participation in this study:

- Investigational drugs must be discontinued no less than 28 days prior to the first dose of study drug;

- Products known to strongly inhibit CYP3A4 metabolism (listed in Appendix 15.4, Table 9) must be discontinued within 5 days prior to Day 1 dosing;
- Products known to strongly induce CYP3A4 metabolism (listed in Appendix 15.4, Table 10) must be discontinued within 28 days prior to Day 1 dosing;
- Products known to strongly inhibit P-gp transporter (listed in Appendix 15.4, Table 11) must be discontinued within 5 days prior to Day 1 dosing;
- Digoxin must be discontinued within 5 days prior to Day 1 dosing;
- Hematopoietic stimulating agents (EPOs, granulocyte colony stimulating factors, thrombopoietins, etc) must be discontinued no less than 28 days prior to the first dose of study drug. [Folic acid 1 mg orally per day is required for all patients. B12 injections are permitted for patients with a prior diagnosis of B12 deficiency syndromes, but must be repleted to stability of the Hb and mean corpuscular volume (MCV) prior to enrollment in the study];
- Anabolic steroids, including testosterone preparations, administered for anemia must be discontinued no less than 28 days prior to the first dose of study drug;
- As the target population for this study consists of transfusion independent patients and transfusion of blood products could confound key endpoints of the study, blood transfusions of any type must be strictly avoided except in cases of compelling medical need. If medical circumstances permit, the Medical Monitor should be contacted for discussion before any transfusions are administered.

Drugs that displace unconjugated bilirubin from albumin (including some common sulfa antibiotics [including sulfamethoxazole/trimethoprim], cephalosporins, salicylates, and aminophylline) should be used with caution with the understanding that patients with elevated levels of unconjugated bilirubin may potentially be at risk for kernicterus syndrome (Strauss, et al. 2006).

**Potential Drug Interactions**

Strong inhibitors of CYP3A4 (listed in Appendix 15.4, Table 9) are not permitted for use with AG-348. Based on modeling of AG-348 metabolism and tolerability data from the Phase 1 MAD study, moderate inhibitors of CYP3A4 do not appear to pose a risk to patients dosed with AG-348.

In-vivo data from the Phase 1 MAD show AG-348 induces CYP3A4 and its own metabolism, presumably via CYP3A4. The use of strong CYP3A4 inducers in combination with AG-348 is expected to reduce AG-348's efficacy. Therefore, administration of strong CYP3A4 inducers (listed in Appendix 15.4, Table 10) is not permitted with AG-348. Corticosteroids may induce CYP3A4. Although the use of corticosteroids is not prohibited, their use should be minimized as much as medically feasible.

Strong inhibitors of drug transport (listed in Appendix 15.4, Table 11) are not permitted for use with AG-348.

Digoxin is not permitted for use with AG-348.

Induction of CYP3A4 by AG-348 is expected to reduce the efficacy of certain sensitive co-medications. Co-administration of AG-348 with the drugs in Appendix 15.4, Table 12 should be done with caution, as their efficacy may be reduced.

Of note, women in the trial utilizing oral contraception must utilize barrier methods as per the Inclusion Criteria 14 (Section 8.2) while taking AG-348.

The expected patient co-medications deferoxamine, deferasirox, deferiprone, and oral penicillin are not expected to interact with AG-348.

### 9.11.3. Allowed Concomitant Therapy

Medications and treatments other than those specified above are permitted during the study. All intercurrent medical conditions will be treated at the discretion of the Investigator according to acceptable local standards of medical care. Subjects may receive analgesics, antiemetics, anti-infectives (including penicillins), and antipyretics as medically indicated and consistent with the guidance in the previous two sections. Patients may continue iron chelation therapy with deferoxamine, deferasirox, or deferiprone. Patients must continue taking at least 1 mg of folic acid for the duration of the study.

All concomitant medications, including transfusions of blood products, procedures performed during the study, including those used to treat AEs, will be recorded on the eCRF.

### 9.11.4. Potential for Phototoxicity

AG-348 may cause sensitivity to direct and indirect sunlight. Patients should be warned to avoid direct sun exposure. When exposure to sunlight is anticipated for longer than 15 minutes, the patient should be instructed to apply factor 30 or higher sunscreen to exposed areas and wear protective clothing and sunglasses.

### 9.11.5. Management of Nausea, Vomiting, and Diarrhea

As the primary objective of this study is to assess the safety and tolerability of up to 24 weeks of AG-348, routine use of prophylactic anti-emetic and antidiarrheal or other classes of medications is prohibited. However, if patients experience AEs relating to nausea, vomiting, or diarrhea these may be treated at the Investigator's clinical discretion with recommended medications as follows:

- Nausea/vomiting: allowed agents include standard clinical dosing with palonosetron (Aloxi), dolasetron (Anzemet), or promethazine (Phenergen). Promethazine is a substrate for CYP2B6, and it is presently unknown if the potential for 2B6 induction after AG-348 dosing could be sufficient to reduce the therapeutic effect of promethazine. Not recommended are aprepitant (Emend; CYP3A4 inhibitor), ondansetron (Zofran; CYP3A4 inducer), chlorpromazine (Thorazine; CYP3A4 inducer), prochlorperazine (Compazine; CYP3A4 substrate, QT prolongation has been reported), and granisetron (Kytril; CYP3A4 substrate, QT prolongation has been reported);
- Diarrhea: recommended management includes standard clinical dosing with Kaopectate or other non-absorbable anti-diarrheals, diphenoxylate/atropine (Lomotil), or loperamide (Imodium). Loperamide is the least preferred choice because it is both

a substrate and inhibitor for CYP3A4, a substrate for CYP2B6, and a substrate for P-gp.

- For the use of any medications not specifically mentioned above the Investigator may confer with the Sponsor's Medical Monitor.

### 9.11.6. Other Restrictions and Precautions

Patients should be advised to refrain from altering their normal exercise routine for the first 28 days of treatment with AG-348 as symptoms of anemia could theoretically temporarily worsen before the eventual potential beneficial effect of AG-348 on the Hb level becomes evident. The elevated blood levels of 2,3-DPG commonly seen in patients with PK deficiency may produce a right shift in the Hb-O2 dissociation curve. The effect of this is to enhance oxygen delivery at the tissue level, thus counteracting to some extent the physiologic consequences of the anemia. AG-348 was shown to produce rapid decreases (within the first 12 hours following a single dose) in 2,3-DPG in two prior clinical trials with healthy adult male and female volunteers. In patients with PK deficiency who have elevated 2,3-DPG levels, it is theoretically possible that decreases in 2,3-DPG that precede correction of the anemia could result in a temporary decrease of oxygen delivery at the tissue level resulting in clinical symptoms (e.g., increased fatigue).

As discussed in Section 5.2.1.1 of this protocol and in the Investigator Brochure, AG-348 has been identified as a histamine H3 receptor antagonist/inverse agonist. No effects of histamine H3 modulation have been observed in safety pharmacology or toxicology studies. Nonetheless, patients should be monitored for potential AEs related to wakefulness and insomnia (Schwartz 2011).

Patients should be advised not to discontinue dosing without first speaking with the treating Investigator—abrupt discontinuation of AG-348 dosing in a patient who experience a substantial increase in Hb may result in withdrawal hemolysis.

# 10. STUDY ASSESSMENTS

## 10.1. Schedule of Assessments

The Schedules of Assessments for this study are provided in Appendix 15.1.

After obtaining written informed consent, patients will undergo screening evaluations. The Screening visit is to be conducted within approximately 42 days prior to first dose of study treatment.

During the Core Treatment Period, patients will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12) and monthly through Week 24 (Weeks 16, 20, and 24). Patients who safely tolerate AG-348 through Week 24 (Core Period) and for whom the Investigator agrees with continuation of AG-348 treatment may be eligible to immediately enter the Extension Period for continued treatment upon agreement of the treating Investigator and the Medical Monitor or Responsible Medical Officer. Study visits for safety and clinical activity assessments will occur approximately every 3 months during the Extension Period. All patients will undergo a follow-up assessment 4 weeks after the last dose of AG-348, regardless of whether this was due to early discontinuation, the last dose in the Core Period for a patient who chooses not to continue in the Extension Period, or the last dose of the Extension Period.

Although *not* encouraged, as a convenience for patients who travel long distances to the study site, in-clinic visits on Day 8 and Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis. For details, please refer to Table 5 in Appendix 15.1 : Schedule of Assessments. For patients having their Day 8 and/or Day 22 visits performed by the primary care physician, the principal investigator will him or herself, or have a qualified research nurse or other designated site staff member, make telephone contact with the patient to inquire about any AEs. These must be recorded as if the patient appeared in the main study center. The Principal Investigator will exercise prudent clinical judgment in determining any clinical course of action to take based on any AEs discovered. These telephone contacts on Day 8 and Day 22 must be explained to the patient in advance and scheduled in advance to maximize the likelihood of successfully making contact. Having in-clinic visits on Days 8 and/or Day 22 performed by the primary care physician will necessitate re-scheduling certain assessments that the primary care physician's office may not be reasonably expected to perform. Table 3 summarizes the details of the re-scheduling of these assessments as described in Table 5 in Appendix 15.1.

During the Extension Period, all scheduled visits must be conducted by the Investigator and at the participating authorized investigative site; local primary care visits will not be allowed.

**Table 3: Summary of Assessments When Day 8 and/or Day 22 In-Clinic Visits Are Performed By Primary Care Physician (Core Period)**

| Day 8 Visit by Primary Care Physician | | | | Day 22 Visit by Primary Care Physician | | | |
|---|---|---|---|---|---|---|---|
| Primary Care Office | Main Study Site | Assessment to Move to Day 15 Main Study Site | Assessments Not Required | Primary Care Office | Main Study Site | Assessment to Move to Day 43 Main Study Site | Assessments Not Required |
| Hematology to central lab | Phone contact with patient | 12-lead ECG | VS; serum chemistry; ETCO | Hematology sample to central lab | Phone contact with patient | 12-lead ECG | VS; serum chemistry; coagulation; haptoglobin; EPO level; carboxyhemoglobin; ETCO; PK/PD |

Abbreviations: ECG = electrocardiogram; EPO = erythropoietin; PK/PD = pharmacokinetics/pharmacodynamics; VS = vital signs

Whenever more than one assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (e.g., VS first, ECG, blood draw). The timing of these assessments should allow the PK blood draw to occur at the exact nominal time. The order of procedures may be revised with prior discussion between Sponsor and Site.

Minor adjustments to the timing, number of planned safety monitoring procedures (e.g., VS, ECG, blood draw), and PK/PD assessments may be made during the course of the study based on collected data to ensure appropriate safety monitoring and will not require a protocol amendment. These minor changes will require prior approval from the Sponsor's Medical Monitor (or Responsible Medical Officer) as well as appropriate documentation in the study records. The addition of new safety monitoring procedures or other assessments will require a protocol amendment.

## 10.2. Informed Consent and Confirmation of Eligibility

A complete description of the study is to be presented to each potential patient and a signed and dated informed consent is to be obtained before any study specific procedures are performed. Separate informed consent forms will be used for the Core and Extension Periods.

Patient's eligibility will be confirmed at Screening and within 24 hours prior to study treatment dose. If a patient is determined to be ineligible, the patient will be excluded from participation.

## 10.3. Demographic Data, Medical and Medication History

Patient demographic data, including gender, date of birth, age, race, and ethnicity, will be obtained at Screening. Collection of demographic data will be modified by country regulatory requirements, as appropriate.

Medication history, including all relevant prior medical history and current medical conditions, will be obtained at the Screening assessment and on Day -1; all concomitant medications administered and procedures conducted within 28 days prior to, or from Screening Visit #1 until the first day of study drug administration, whichever interval is longer, should be reported in the source documentation and eCRF. Investigators will be asked to provide information on the patient's history of any medical diagnoses (e.g., iron overload) and surgical procedures (e.g.,

splenectomy, cholecystectomy) pertaining to their diagnosis of PK deficiency and prior available complete blood counts (CBCs) over the preceding 6 months and transfusion history over the preceding 12 months prior to the date of signing informed consent.

## 10.4. PKR Enzymatic Assay and PKR Genotyping

Assessments for PKR enzymatic activity and PKR genotyping will be performed at Screening only for confirmation of study eligibility. PKR enzymatic assays will be conducted at [redacted] or any participating investigative site's local hematology laboratory. PKR genotyping will be conducted at [redacted]

## 10.5. Safety Assessments

### 10.5.1. Physical Examination, Height, and Weight

A complete physical examination (including neurological examination; genital and rectal examinations will be performed at the discretion of the Investigator) will be obtained at Screening, Baseline, Week 12, and the Follow up Visit (Week 28). The neurological examination must include an assessment of general orientation and mental status including level of alertness (assess as normal or abnormal and specify any abnormality). Limited focused physical examinations will be performed at all other visits during the Core Period. Focused physical examinations, including neurological examination, will continue every 3 months during the Extension Period; a complete physical examination will be performed at Month 30. Any findings will be recorded on the eCRF. Height will be collected at Screening only.

### 10.5.2. Vital Signs

Vital signs, including systolic and diastolic BP, heart rate, respiratory rate, and temperature, will be obtained according to the according to the Schedules of Assessments (Appendix 15.1). Vital sign measurements will be repeated if clinically significant or machine/equipment errors occur. Out of range BP or heart rate measurements will be repeated at the Investigator's discretion. Any confirmed, clinically significant vital sign measurements will be recorded as AEs.

### 10.5.3. Electrocardiogram

A 12-lead ECG will be obtained according to the Schedules of Assessments (Appendix 15.1). The ECGs will be measured using an ECG machine that reports the heart rate and PR, QRS, QT, QTcB (Bazett correction formula; may also be calculated), and QTcF intervals (may also be calculated). Only QTcF (not QTcB) will be used for determination of eligibility.

The 12-lead ECGs should be obtained following 5 minutes of recumbency. ECGs will be repeated if clinically significant abnormalities are observed, if artifacts are present, or if machine/equipment errors occur. Any confirmed, clinically significant ECG findings will be recorded as AEs.

### 10.5.4. DXA Scans

DXA scans (hip and spine) will be performed at Screening to obtain T and Z scores and bone mineral density that will serve as a baseline measure for all enrolling patients. An additional DXA scan for the Core Period will be conducted in the interval between Week 24 and Week 28,

and scans will be conducted at Months 18 and 30 during the Extension Period as indicated in the Schedules of Assessments (Appendix 15.1). All redacted DXA scan reports must be held at the study site and will be collected by the Sponsor.

### 10.5.5. Safety Laboratory Assessments

#### 10.5.5.1. Hematology, Serum Chemistry, Coagulation Studies, and Urinalysis

Laboratory values obtained prior to Screening and RBC antibodies obtained at Screening will be performed at a local laboratory. On-study clinical laboratory evaluations are to be performed by a central laboratory. If Investigators believe that it is clinically indicated to obtain safety laboratory results from their own local laboratories on the day of the subject's visit, and before the results are returned from the designated central laboratory, they are free to exercise their discretion to do so. Investigators should be aware that since the Hb level is a secondary endpoint of the study, it behooves them to minimize blood volumes drawn, and samples for the central lab must still be collected, since it will serve as the official lab result for this study.

Clinical laboratory evaluations are to be collected according to the Schedules of Assessments (Appendix 15.1). In addition, all clinically significant laboratory abnormalities noted on testing will be followed by repeat testing and further investigated according to the judgment of the Investigator. Please note that serum estradiol, free and total testosterone, and CBC will be collected in the AM at any 2 time points during Screening at least 2 days apart in addition to Baseline/Day 1 (total of 3 time points prior to Day 1 dosing).

The following safety laboratory parameters are to be determined:

| | |
|---|---|
| **Hematology**: | HCT, Hb, RBC count, absolute reticulocyte count, percent reticulocyte count, MCV, mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count with automated (or manual, as indicated) differential, ANC, and absolute lymphocyte count (ALC), and platelet count. In the event that the designated central laboratory for the study is unable to provide a valid result for any specific component of the defined CBC for a specific patient, the site may be asked to have the test performed at their local laboratory. The result of the local CBC will be entered into the study database along with the local normal reference range. G6PD (may be conducted at Mayo Medical Laboratories [Rochester, MN] or any participating investigative site's local hematology laboratory) and RBC antibody screen will be performed at Screening only |
| **Other** | EPO, Hp, COHb, ETCO (at a subset of sites during the Core Period), hepcidin, 25-hydroxy vitamin D2 and D3.<br><br>**Measurement of ETCO by CoSense® Monitor**<br><br>Assessment of ETCO as measured by the CoSense® Monitor device will be performed at a limited number of participating study sites on consenting patients during the Core Period. The patient will be fitted |

| | |
|---|---|
| | with small single-use, disposable nasal prongs attached to a cannula that is connected to the CoSense® monitor. The patient breathes normally and the CoSense® monitor collects and analyzes the patient's exhaled air within approximately 5 minutes, generating a measurement of end tidal carbon monoxide in parts per million (ppm). This value is entered into the clinical database. The assessment will be performed according to the schedule detailed in Section 15.1, Table 5. Carbon monoxide is produced in a 1:1 ratio from the breakdown of heme and has been correlated with the rate of hemolysis (Christensen, et al. 2015; Christensen, et al. 2016). |
| **Serum Chemistry:** | alkaline phosphatase (ALP), sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide ($CO_2$) or bicarbonate, albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, LDH, ALT, AST, total bilirubin, indirect bilirubin, (and estimated creatinine clearance or GFR for screening only, as appropriate). |
| **Sex Hormones**: | testosterone (total and free), estrone, and estradiol. FSH will only be performed at Screening for female patients only for confirmation of post-menopausal status. |
| **Bone Turnover** | serum osteocalcin-N-mid and CTX. |
| **Lipids** | total cholesterol, HDL-C, triglycerides. |
| **Iron Panel** | iron, total iron-binding capacity (TIBC), transferrin saturation, ferritin. |
| **Coagulation Studies:** | fibrinogen, aPTT, INR. |
| **Urinalysis**: | color and appearance, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, pregnancy screen (dipstick), occult blood. Microscopic inspection of sediment will only be performed for cause or to investigate an abnormal dipstick finding per the Investigator's discretion. |

#### 10.5.5.2. Screening Serology

A blood sample for serology, including HBsAg, HCV Ab screen, and HIV1 and HIV2 Ab, is to be collected from all patients at Screening.

### 10.5.6. Menstrual Cycle Diary

Menstruating female patients will be required to fill out a paper-based menstrual cycle diary each month in order to monitor any changes. Diaries will be dispensed and collected as indicated in

the Schedules of Assessments (Appendix 15.1). Patients will record the start date, stop date, and any notable characteristics of each menstrual cycle.

### 10.5.7. Adverse Events

Each patient will be carefully monitored for the development of any AEs throughout the study from signing of the informed consent through all scheduled study follow-up visits, or withdrawal of consent, whichever occurs first. In addition, SAEs that are assessed as possibly or probably related to study treatment that occur > 30 days post-treatment also are to be reported.

Adverse events will be evaluated by the Investigator and recorded as described in the Schedules of Assessments. On dosing visits, all AEs (elicited and spontaneously reported) will be continuously evaluated by the Investigator and recorded. At any non-dosing day visit, AEs will be evaluated by the Investigator and recorded. Any AEs already documented at a previous assessment and designated as ongoing will be reviewed at subsequent visits or assessment time points as necessary. If these AEs have resolved, this will be documented.

All AEs will be graded using the CTCAE grading system (Appendix 15.3 ).

Complete details on AE monitoring are provided in Section 11.

## 10.6. Pharmacokinetic Assessments

The first approximately 10 patients treated in the Core Period, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 15.1, Table 7. The remainder of treated patients will undergo limited PK sampling as detailed in Appendix 15.1, Table 8. The in-clinic visit on Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis. In this instance, PK sampling will not be required on Day 22. (Additional details regarding Day 8 and Day 22 visits performed by the patient's primary care physician can be found in Table 5 in Appendix 15.1: Schedule of Assessments: Core Period.) During the Extension Period, predose PK samples will be drawn for the measurement of trough levels of AG-348 and AGI-8702 at each study visit (every 3 months; see Appendix 15.1, Table 6).

The collection times for post-dose PK samples will start from the time that dosing is completed. (For example, a PK draw at 30 minutes will be collected 30 minutes after the last capsule has been ingested.) The completion time of each dose will be recorded.

Procedures for sample collection and processing will be provided in a separate study manual. The actual time point of each sample collection will be recorded.

Samples for PK and PD assessments may be retained for up to 2 years from collection.

## 10.7. Pharmacodynamic Assessments

The first approximately 10 patients treated in the Core Period, contingent on clinical site feasibility, will undergo extensive PD sampling for 2,3-DPG and ATP as detailed in Appendix 15.1, Table 7. The remainder of treated patients will undergo limited PD for 2,3-DPG and ATP sampling as detailed in Appendix 15.1, Table 8. During the Extension Period, predose PD samples will be drawn for the measurement of trough levels of 2,3-DPG, ATP, [redacted] at each study visit (every 3 months; see Appendix 15.1, Table 6).

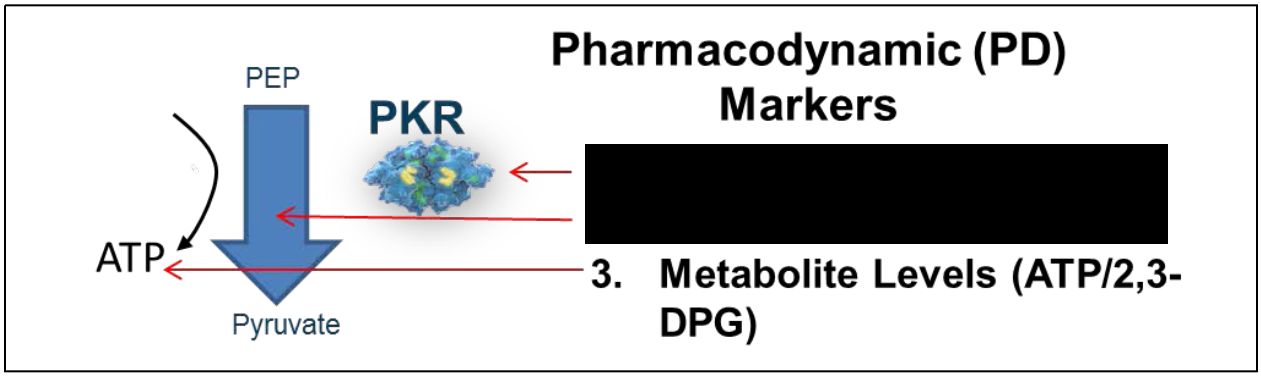
The collection times for post-dose PD samples will start from the time that dosing is completed. (For example, a PD draw at 30 minutes will be collected 30 minutes after the last capsule has been ingested.) The completion time of each dose will be recorded.

Procedures for sample collection and processing will be provided in a separate study manual. The actual time point of each sample collection will be recorded.

Pharmacodynamic assessments during the Core Period will include 2,3-DPG, ATP, [redacted] The in-clinic visit on Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis. In this instance, PD sampling will not be required on Day 22. (Additional details regarding Day 8 and Day 22 visits performed by the patient's primary care physician are stated in Table 5 in Appendix 15.1: Schedule of Assessments.)

Figure 4 provides a brief schematic outlining the PKR reaction and how each of these PD assessments fits into a complete mechanistic understanding of the action of AG-348.

**Figure 4: PKR Enzymatic Reaction**




The PKR enzyme catalyzes the PEP to pyruvate reaction, with concomitant formation of ATP.

- Binding of AG-348 to the PKR tetramer can be assessed through an ex-vivo biochemical assay of cell lysates from AG-348 treated patients. Because WBCs contain a high level of pyruvate kinase from a non-PKR pyruvate kinase isoform, WBCs are first removed by filtration before the purified red cells are frozen. [redacted] It has been reported in the literature that there may be compensatory expression of PKM2 in the RBCs of some patients with PKR deficiency (Black, et al. 1979; Kahn, et al. 1975; Rijksen, et al. 1990). Therefore, levels of PKM2 and appropriate reference proteins (e.g. actin, GAPDH) may be evaluated in these whole blood samples.
- [redacted]

[redacted]

- AG-348 target engagement [redacted] has been shown in preclinical models and healthy volunteer clinical studies to result in accumulation of ATP and depletion of the upstream metabolite 2,3-DPG. Therefore, levels of these metabolites will be measured by mass spectrometry in frozen whole blood samples.

The first approximately 10 patients treated during the Core Period, contingent on clinical site feasibility, will undergo extensive PD sampling as detailed in Appendix 15.1, Table 7. The remainder of treated patients will undergo limited PD sampling as detailed in Appendix 15.1, Table 8. The collection times for post-dose PD samples will start from the time that dosing is completed. (For example, a PD draw at 30 minutes will be collected 30 minutes after the last capsule has been ingested.) The completion time of each dose will be recorded.

Blood samples will be stored at the site and regularly transported at -80°C ±10 C to the bioanalytical laboratory for analysis. Procedures for sample collection and processing will be provided in a separate study manual.

## 10.8. Ordering of Blood Sample Collection

When more than 1 blood sample is collected at the same nominal time, the samples will be collected in the following order:

1. Safety laboratory assessments
2. PD (2,3 DPG, ATP)

[redacted]

4. PK

[redacted]

## 10.9. Sample Processing, Storage, and Shipment

Instructions for the processing, storage and shipment of all study samples for central analysis will be provided in a separate study manual.

# 11. ADVERSE EVENTS

Monitoring of AEs will be conducted throughout the study. Adverse events and SAEs will be recorded in the source documentation and eCRF from time of the signing informed consent through the Follow-up Visits for randomized patients. All AEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

All SAEs will be followed through 30 days after the last dose of study treatment or until the SAE has resolved. Any SAEs that are assessed as possibly or probably related to study treatment that occur > 30 days post-treatment also are to be reported.

## 11.1. Definition of Adverse Events

### 11.1.1. Adverse Event

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An AE can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

### 11.1.2. Suspected Adverse Reaction

A suspected adverse reaction is any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of expedited safety reporting, 'reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the AE.

### 11.1.3. Unexpected Adverse Event

An unexpected AE is one for which the nature or severity of the event is not consistent with the applicable product information, e.g., the Investigator's Brochure.

### 11.1.4. Serious Adverse Event

An AE or suspected adverse reaction is considered serious (SAE) if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death;
- Life-threatening. Life-threatening means that the patient was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form;
- In-patient hospitalization or prolongation of existing hospitalization. Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered AEs if the illness or disease existed before the patient was enrolled in the study, provided that it did not deteriorate in an unexpected manner during the study (e.g., surgery performed earlier than planned);

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- Congenital anomaly/birth defect;
- Important medical event. An important medical event is an event that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the patient or patient and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions for SAEs. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalization, or the development of drug dependency or drug abuse.

#### 11.1.4.1. Potential Severe Drug-Induced Liver Injury

The document entitled FDA Guidance for Industry, Drug-Induced Liver Injury: Premarketing Clinical Evaluation (FDA 2009) provides guidance on how the measurement of various laboratory parameters may be used to assess a given drug's potential to cause severe liver injury (i.e., irreversible liver failure that is fatal or requires liver transplantation). Such cases are suggested by the following three components:

1. The drug causes hepatocellular injury, generally shown by a higher incidence of 3-fold or greater elevations above the ULN of ALT or AST than the (non-hepatotoxic) control drug or placebo;
2. Among trial patients showing such aminotransferase elevations, often with aminotransferases much greater than 3×ULN, one or more also show elevation of serum total bilirubin to > 2×ULN, without initial findings of cholestasis (elevated serum ALP);
3. No other reason can be found to explain the combination of increased aminotransferases and total bilirubin, such as viral hepatitis A, B, or C; pre-existing acute liver disease; or another drug capable of causing the observed injury.

Clinical safety laboratory results compatible with the definition of drug-induced liver injury (DILI) stated above must be repeated for confirmation as soon as possible, and if confirmed, will be scored as an unacceptable AE and reported to FDA as a serious unexpected AE.

## 11.2. Procedures for Reporting Adverse Events and Serious Adverse Events

Each patient must be carefully monitored for the development of any AEs. This information should be obtained in the form of non-leading questions (e.g., "How are you feeling?") and from signs and symptoms detected during each examination, observations of study personnel, and spontaneous reports from patients.

All AEs (serious and non-serious) spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures will be recorded in the source documentation and eCRF. Any clinically relevant deterioration in laboratory assessments or other clinical findings is considered an AE and must be recorded in the appropriate source documentation and eCRF. Treatment-emergent

abnormal clinical laboratory results should generally be reported as AEs if there are accompanying symptoms; if additional diagnostic evaluations or medical (including drug therapy) or surgical interventions are undertaken; if a change in study drug dosing or study drug discontinuation is required; or, if the laboratory result is considered clinically significant by the Investigator. Although it is an objective of this study to determine if treatment with AG-348 results in increased Hb levels in patients with PK deficiency, overshoot of the Hb level above the ULN by gender is not recommended and should be reported as an AE, graded per the CTCAEv4.03. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

All SAEs that occur during the course of the study must be promptly reported by the Investigator to [redacted] Global Safety and Pharmacovigilance (see below). Deaths and AEs assessed as life-threatening are to be reported immediately and SAEs that meet other criteria are to be reported within 24 hours from the point in time when the Investigator becomes aware of the SAE. All SAEs must be reported whether or not they are considered causally related to AG-348. Serious AE forms will be completed and the information collected will include subject number, a narrative description of the event, and an assessment by the Investigator as to the severity of the event and relatedness to study drug. Follow-up information on the SAE may be requested by the Sponsor or Medical Monitor.

**Contact Information:**

[redacted] **Safety Management**

SAE Hotline (United States): [redacted]
SAE Fax (United States): [redacted]

SAE Hotline (France): [redacted]
SAE Fax (France): [redacted]

SAE Hotline (Netherlands): [redacted]
SAE Fax (Netherlands): [redacted]

SAE Hotline (Italy): [redacted]
SAE Fax (Italy): [redacted]

SAE Hotline (United Kingdom): [redacted]
SAE Fax (United Kingdom): [redacted]

E-mail: [redacted]

If there are serious, unexpected adverse drug reactions associated with the use of AG-348, the Sponsor will notify the appropriate regulatory agency(ies) and all participating Investigators on an expedited basis. The local IRB/IEC will be promptly notified based on local regulations where required by the IRB/IEC of all serious, unexpected adverse drug reactions involving risk to human subjects.

All AEs, whether serious or not, will be described in the source documents in the database. All new events, as well as those that worsen in intensity or frequency relative to baseline, which occur after signing the informed consent through the final Follow-up Visit (Day 29 ± 3 days) must be recorded. Adverse events that are ongoing at the time of treatment discontinuation

should be followed up to 30 days after the last dose of study treatment. All SAEs will be followed up to 30 days after the last dose of study treatment, or until the SAE has resolved.

Information to be reported in the description of each AE includes:

- A medical diagnosis of the event (if a medical diagnosis cannot be determined, a description of each sign or symptom characterizing the event should be recorded);
- The date of onset of the event;
- The date of resolution of the event;
- Whether the event is serious or not;
- Intensity of the event (see below for definitions);
- Relationship of the event to study treatment (see below for definitions);
- Action taken: none; change in the study drug administration (e.g., temporary interruption in dosing); drug treatment required; non-drug treatment required; hospitalization or prolongation of hospitalization required (complete SAE page); diagnostic procedure performed; patient discontinued from the study (complete End of Study visit);
- Outcome: patient recovered without sequelae; patient recovered with sequelae; event ongoing; patient died (notify the Medical Monitor immediately, and complete the SAE form).

**Intensity** of all AEs will be graded according to the NCI CTCAE Version 4.03 (Appendix 15.3).

**Relationship** to study drug administration will be determined by the Investigator according to the following criteria:

- **Not Related**: Exposure to the study treatment did not occur, or the occurrence of the AE is not reasonably related in time, or the AE is considered unlikely to be related to the study treatment.
- **Possibly Related**: The study treatment and the AE were reasonably related in time, and the AE could be explained equally well by causes other than exposure to the study treatment. An attribution of possibly related means that there are facts in evidence to suggest a possible relationship.
- **Probably Related**: The study treatment and the AE were reasonably related in time, and the AE was more likely explained by exposure to the study treatment than by other causes, or the study treatment was the most likely cause of the AE. An attribution of probably related means that there are facts in evidence to suggest a probable relationship.

For the purpose of safety analyses related to final database review, all AEs that are classified as possible or probable will be considered treatment-related AEs.

## 11.3. Pregnancy Reporting

Pregnancy is neither an AE nor an SAE, unless a complication relating to the pregnancy occurs (e.g., spontaneous abortion, which may qualify as an SAE). However, any pregnancy in a participating female patient or a female partner of a participating male patient that occurs during this study or within 30 days following the last dose of AG-348 must be reported to the Sponsor or Medical Monitor within 30 days of being notified of the pregnancy. The Investigator must follow up and document the course and outcome of all pregnancies even if the patient was discontinued from the study or if the study has finished. The female patient or partner of a male patient should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus. Monitoring should continue until conclusion of the pregnancy.

All outcomes of pregnancy must be reported by the Investigator to the Sponsor or Sponsor's designee on a Pregnancy Outcome Report form within 30 days after he/she has gained knowledge of the delivery or elective abortion.

Any SAE that occurs during pregnancy must be recorded on the SAE report form (e.g., maternal serious complications, spontaneous or therapeutic abortion, ectopic pregnancy, stillbirth, neonatal death, congenital anomaly, or birth defect) and reported within 24 hours in accordance with the procedure for reporting SAEs.

All patients, male and female, must agree to use effective contraception during the entire study and for 30 days following the last dose of AG-348. Abstinence is an acceptable method only when this is in line with the normal life style of the patient, meaning that the patient plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence, e.g. calendar, sympathothermal and post-ovulation methods, and withdrawal are not acceptable methods of contraception.

# 12. STATISTICAL METHODS

The primary objective of this Phase 2 study is to evaluate the safety and tolerability of up to 24 weeks of AG-348 in patients with PK deficiency. Therefore, analyses will be primarily descriptive in nature; no formal hypothesis testing will be conducted. All analyses will be conducted separately within each dose arm, or pooled where appropriate. Analyses of safety and of indicators of clinical activity will be conducted for the Core Period, Extension Period, and overall, if applicable. For the Core Period, the data to be analyzed will include all collected data through 24 weeks of treatment for patients who directly enter the Extension period. For patients who do not enter the Extension Period, the analyses will include all collected data through the duration of treatment (24 weeks or less) plus 4 week follow up data. For patients who move directly from the Core to the Extension Period, the 4 week follow up data will be analyzed as part of the Extension Period.

## 12.1. Sample Size Estimation

Due to the rare disease setting, the minimal sample size in each dose arm may be determined by feasibility. In order to evaluate the primary objective of safety and tolerability of AG-348 in Arm 1 and Arm 2, up to a maximum of 25 patients may be randomized onto each arm. The actual number of patients enrolled into Arms 1 and 2 will depend on the safety reviews and decisions made by the DRT. In addition, up to 25 additional patients may be enrolled to evaluate an additional dose arm (Arm 3; see Section 7.1). As for Arms 1 and 2, the actual enrollment in a potential Arm 3 will depend on the safety reviews and decisions made by the DRT. Therefore, up to approximately 75 total patients may be enrolled in this study across 2 to 3 dose arms.

Table 4 provides the probability within a dose arm of detecting 1 or more AEs with varying sample size and the true underlying AE rates.

**Table 4: Sample Size Estimation**

| Sample Size | True Underlying AE Rate | | |
|---|---|---|---|
| | **15%** | **10%** | **5%** |
| 5 | 56% | 41% | 23% |
| 10 | 80% | 65% | 40% |
| 15 | 91% | 79% | 54% |
| 25 | 98% | 93% | 72% |

## 12.2. Populations for Analysis

The following patient populations (i.e., analysis sets) will be evaluated and used for presentation of the data:

- Safety Analysis Set: All patients who are enrolled and receive any dose of study treatment. The Safety Analysis Set will be the primary set for the analysis of safety

data. Patients will be classified according to the actual treatment received and whether they underwent an intra-patient dose escalation, where treatment received is defined as the assigned treatment if it is received at least once, or as the first treatment received if assigned treatment is never received. Unless otherwise stated, the Safety Analysis Set will be the default analysis set for all data analyses.

- Pharmacokinetic Analysis Set: All patients in the Safety Analysis Set with sufficient plasma sample data to assess PK parameters. Results of the potential PD activity of AG-348 will also be based on the PK analysis set.
- Efficacy Analysis Set: All patients who are enrolled and achieve at least 50% compliance with their assigned dose intensity for at least 4 weeks of continuous dosing. Additional efficacy analyses may be performed on subsets of patients with other degrees of compliance with their assigned dose intensity and/or duration of dosing. The Efficacy Analysis Set will be the primary set for the analysis of preliminary clinical activity data. Patients will be classified according to the actual treatment received and whether they underwent an intra-patient dose escalation, where treatment received is defined as the assigned treatment if it is received at least once, or as the first treatment received if assigned treatment is never received.

[redacted] If such analyses are performed, they will be described in a separate PK Statistical Analysis Plan (SAP) and may be reported separately in a stand-alone report.

## 12.3. Procedures for Handling Missing, Unused, and Spurious Data

No imputation will be performed for missing data elements. When summarizing AE data, partial dates will be imputed as described in the SAP. Additional rules addressing the handling of missing data will be detailed in the SAP.

## 12.4. Interim Analysis

No formal statistical interim analysis will be conducted. Safety data will be reviewed on an ongoing basis by the DRT, who will meet to review safety, PK, PD, and preliminary clinical activity data at regular intervals (approximately every 6 weeks during the Core Period and approximately every 3 months during the Extension Period once all patients have completed the Core Period) throughout the duration of the Core Period. The DRT's decisions to suspend, terminate, or open a potential third dosing arm, or re-assign patients' dosing in a terminated arm to match the dose and schedule of another arm of the study will be based on the totality of the data including, safety, PK, PD, and preliminary clinical activity (e.g., changes in Hb levels).

Additional interim reviews of data may be conducted to support decision making concerning the current clinical study, the sponsor's development programs in general, or in case of any safety concerns.

## 12.5. Statistical Methodology

### 12.5.1. General Methods

This study will be primarily descriptive in nature; therefore, there will be no formal hypothesis testing. Summaries will be produced for disposition, baseline disease characteristics and demographic data including genotype, safety measurements, PK, PD parameters and indicators of clinical activity. Data from each AG-348 dose group will be analyzed separately, and pooled across all dose groups where appropriate. All data will also be listed by individual patient.

Categorical variables will be summarized by frequency distributions (number and percentages) and continuous variables will be summarized by descriptive statistics (mean, standard deviation [SD], median, minimum, and maximum).

The study database will be locked and statistical analysis will be performed after all patients have completed the Core Period. The results of this analysis will be presented in a clinical study report (CSR). Additional data collected during the Extension Period after the CSR database lock will be analyzed for inclusion in a subsequent CSR addendum.

### 12.5.2. Disposition

A summary of the disposition of patients will be presented, including the number enrolled, the number treated, and the reasons for study discontinuation. Entry criteria and protocol deviations will be listed.

### 12.5.3. Exposure and Safety Analyses

Patients will receive multiple PO doses of AG-348 over a 24-week treatment period. The actual dose and duration in days of AG-348, as well as the dose intensity (computed as the ratio of actual dose received and actual duration) and the relative dose intensity (computed as the ratio of dose intensity and planned dose received/planned duration) will be listed and summarized using descriptive statistics by dose arm.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and the incidence of TEAEs (new or worsening from baseline) will be summarized by primary system organ class (SOC), preferred term, CTCAE Version 4.03 severity, outcome, action taken with study drug, and relationship to the study drug by dose group. Separate summaries will be produced for all TEAEs, treatment-related AEs (those considered by the Investigator as at least possibly drug related), SAEs, discontinuations due to AEs, and AEs ≥ Grade 3 severity. Individual patient listings will be provided for deaths, SAEs, AEs leading to interruption and/or reduction of study drug dose, and AEs leading to discontinuation of treatment.

Analyses of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined as any AE with onset beginning at the day of first administration of study drug, throughout the treatment period until 30 days after the last dose of study drug, or any event that was present at baseline but worsened in intensity or was subsequently considered drug-related by the Investigator through the end of the study.

Descriptive statistics will be provided for clinical laboratory values (e.g., hematology, serum chemistry, coagulation studies, urinalysis) and VS data, presented as both actual values and changes from baseline relative to each on-study evaluation. Shift analyses will be conducted for

selected laboratory parameters based on the baseline CTCAE grade to maximum CTCAE grade. Where applicable CTCAE terms do not exist, a grading system based on the upper and/or lower limits of normal will be used to classify the results.

No formal hypothesis-testing analysis of AE incidence rates will be performed.

Electrocardiogram analyses will include individual patient listings and summaries of abnormal and clinically significant ECG results. Actual values and changes from baseline in PR, QRS, and heart-rate corrected QT interval (QTc) intervals will be summarized by visit and dose arm. Full details of the QTc analysis including correction methods used will be described in the SAP.

Data collected from the menstrual diaries such as the start and stop dates of the menses and the patient reported characteristics of the menses will be presented in a by-patient listing. Additional descriptions of the data may also be performed.

Concomitant medications will be coded using the WHO Drug Dictionary. Concomitant medications prior to and after the start of the study drug will be listed by patient, and summarized by ATC term and dose arm.

### 12.5.4. Pharmacokinetic Analyses

Descriptive statistics will be used to summarize PK parameters for the parent compound AG-348 and the metabolite AGI-8702 for each dose group, and where appropriate, for the entire population. Pharmacokinetic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum and maximum, geometric mean, and geometric coefficient of variation. PK analyses will be described in a separate PK SAP.

### 12.5.5. Pharmacodynamic Analyses

Descriptive statistics will be used to summarize PD parameters for 2,3-DPG and ATP for each dose group, and where appropriate for the entire population. Pharmacodynamic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation %. PD analyses will be described in a separate PD SAP.

### 12.5.6. Aromatase Hormone Analysis

The analyses of serum sex hormones will use appropriate graphic displays and statistical analyses to evaluate patient change from baseline for each parameter. These analyses will include summaries of actual values and change from baseline using appropriate descriptive statistics (mean, SD, median, min and max) as described above.

[redacted] These analyses will present information by each dose arm, and analyses of a pooled AG-348 cohort. Additional details regarding these analyses will be provided in the SAP.

### 12.5.7. Clinical Activity

Details on analyses to evaluate indicators of potential clinical activity of AG-348 in patients with PK deficiency will be described in the SAP. These will include changes in Hb, HCT, reticulocyte count, Hp, COHb, ETCO (Core Period only), LDH, total and indirect bilirubin, EPO, hepcidin,

ferritin, and transferrin saturation (serum iron/iron binding capacity). Characterization of Hb response which will include, but is not limited to percent of patients with increase in Hb, time to Hb response, and duration of Hb response will be explored.

[redacted]

## 12.6. Procedures for Reporting Deviations to Original Statistical Analysis Plan

All deviations from the original SAP will be provided in the final CSR.

# 13. ADMINSTRATIVE REQUIREMENTS

## 13.1. Good Clinical Practice

The study will be conducted in accordance with the ICH for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s). The Investigator will be thoroughly familiar with the appropriate use of the study drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study, and retained according to the appropriate regulations.

## 13.2. Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki (Appendix 15.5).

The Investigator must obtain IRB approval for the investigation and must submit written documentation of the approval to the Sponsor before he or she can enroll any patient into the study. The IRB will review all appropriate study documentation in order to safeguard the rights, safety, and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, Investigator's Brochure, informed consent, advertisements (if applicable), written information given to the patients, annual progress reports, and any revisions to these documents will be provided to the IRB. The IRB is to be notified of any amendment to the protocol in accordance with local requirements. Progress reports and notifications of serious unexpected adverse drug reactions are to be provided to the IRB according to local regulations and guidelines.

## 13.3. Patient Information and Informed Consent

The Investigator or trained designee will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk, and benefit of the study. Patients must also be notified that they are free to discontinue from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

After the study has been fully explained, written informed consent will be obtained from the patient prior to study participation.

The patient's signed and dated informed consent must be obtained before conducting any study-related procedures. The Investigator must maintain the original, signed consent form. A copy of the signed form must be given to the patient.

The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

## 13.4. Patient Confidentiality

In order to maintain patient privacy, all source documents, study drug accountability records, study reports and communications will identify the patient by the assigned patient number. The

Investigator will grant monitor(s) and auditor(s) from the Sponsor or its designee and regulatory authority(ies) access to the patient's original medical records for verification of data gathered on the source documents and to audit the data collection process. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

## 13.5. Protocol Compliance

The Investigator will conduct the study in compliance with the protocol. Modifications to the protocol should not be made without agreement of both the Investigator and the Sponsor. Changes to the protocol will require written IRB approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to patients. The IRB may provide, if applicable, where regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval/favorable opinion of the IRB. The Sponsor or designee will submit all protocol modifications to the regulatory authority(ies) in accordance with the governing regulations.

When immediate deviation from the protocol is required to eliminate an immediate hazard(s) to patients, the Investigator will contact the Sponsor's Medical Monitor (or Responsible Medical Officer), if circumstances permit, to discuss the planned course of action. Any departures from the protocol must be fully documented in the source documents/database.

## 13.6. Data Management

A paper or eCRF will be completed for each patient or an electronic data capture (EDC) system will be used. The EDC system (Medidata Rave®) is a software tool designed to ensure quality assurance and facilitate data capture during clinical trials. Through a system regulated workflow that includes barcode scanning and interfaces to medical equipment to avoid manual data entry, study operations performance is controlled and captured in real time. The system is fully Code of Federal Regulations (CFR) 21 Part 11 compliant. Source documentation supporting the data should indicate participation in the study and should document the dates and details of study procedures, AEs, and patient status. The Investigator, or trained designee should complete and the Investigator should verify the source documents as the information is collected. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data. The Investigator will ensure the accuracy, completeness, and timeliness of the data reported to the Sponsor. Data collection processes and procedures will be reviewed and validated to ensure completeness, accuracy, reliability, and consistency. A complete audit trail will be maintained of all data changes. The Investigator or designee will cooperate with the Sponsor's representative(s) for the periodic review of study documents to ensure the accuracy and completeness of the data capture system at each scheduled monitoring visit. Electronic consistency checks and manual review will be used to identify any errors or inconsistencies in the data. This information will be provided to the respective study sites by means of electronic or manual queries. The Investigator or designee will prepare and maintain adequate and accurate study documents (medical records, ECGs, AE, and concomitant medication reporting, raw data collection forms, etc.) designed to record all observations and other pertinent data for each patient receiving study treatment. The Investigator will allow

Sponsor representatives, contract designees, authorized regulatory authority inspectors, and the IRB to have direct access to all documents pertaining to the study.

## 13.7. Source Document/Case Report Form Completion

Source documents will be completed for each study patient. It is the Investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the patient's source document. The source document should indicate the patient's participation in the study and should document the dates and details of study procedures, AEs and patient status.

The Investigator, or designated representative, should complete the source document as soon as possible after information is collected, preferably on the same day that a study patient is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.

The Investigator will sign and date each required assessment for all study patients. The Investigator will retain all completed source documents.

## 13.8. Direct Access to Source Data

The study will be monitored by the Sponsor or its designee. Monitoring will be done by personal visits from a representative of the Sponsor (site monitor) and will include on-site review of the source documents for completeness and clarity, cross-checking with source documents, and clarification of administrative matters will be performed. The review of medical records will be performed in a manner to ensure that patient confidentiality is maintained.

The site monitor will ensure that the investigation is conducted according to protocol design and regulatory requirements by frequent communications (letter, telephone, e-mail, and fax).

All unused study drug and other study materials are to be returned to the Sponsor or designee after the study has been completed and the database has been locked.

Regulatory authorities, the IRB, and/or the Sponsor's clinical quality assurance group or designee may request access to all source documents, database, and any other applicable study documentation for an on-site audit or inspection. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities.

## 13.9. Record Retention

The Investigator will maintain all study records according to ICH-GCP and applicable regulatory requirement(s). Records will be retained for at least 2 years after the last marketing application approval or 2 years after formal discontinuation of the clinical development of the investigational product or according to applicable regulatory requirement(s). If the Investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility. The Sponsor must be notified in writing if a custodial change occurs.

## 13.10. Liability and Insurance

The Sponsor has subscribed to an insurance policy covering, in its terms and provisions, its legal liability for injuries caused to participating persons and arising out of this research performed

strictly in accordance with the scientific protocol as well as with applicable law and professional standards.

## 13.11. Publication of Study Findings and Use of Information

All information regarding AG-348 supplied by the Sponsor or designee to the Investigator is privileged and confidential information. The Investigator agrees to use this information to accomplish the study and will not use it for other purposes without consent from the Sponsor. It is understood that there is an obligation to provide the Sponsor with complete data obtained during the study. The information obtained from the clinical study will be used towards the development of AG-348 and may be disclosed to regulatory authority(ies), other Investigators, corporate partners, or consultants as required.

# 14. LIST OF REFERENCES

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# 15. APPENDICES

## 15.1. Schedules of Assessments

### Table 5: Schedule of Assessments: Core Period

| **Timing** | **Pre-Treatment** | | **Month 1** | | | | **Months 2 and 3** | | | **Months 4, 5 and 6** | | | **Follow Up**[1] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | | **Baseline / D1** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day** | **-42 to -1** | **-40 to -1**[2] | **1** | **8**[3] | **15** | **22**[3] | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window** | | | | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| Written Informed Consent | X | | | | | | | | | | | | |
| PK enzyme assay (confirmation of PK deficiency)[4] | X | | | | | | | | | | | | |
| PKR Genotype (for randomization) | X | | | | | | | | | | | | |
| UGT1A1 Genotype | X | | | | | | | | | | | | |
| Demographics | X | | | | | | | | | | | | |
| Medical/Surgical History (General and PK deficiency-specific)[5] | X | | | | | | | | | | | | |
| Medication History | X | | | | | | | | | | | | |
| Transfusion History | X | | | | | | | | | | | | |
| Confirmation of Vaccinations (Splenectomized Patients) | X | | | | | | | | | | | | |
| Physical Examination[6]/ Height[6] and Weight | X | | X | | X | | | X | X | X | X | X | X |
| Performance Status | X | | X | | X | | | X | X | X | X | X | X |

| **Timing** | **Pre-Treatment** | | **Month 1** | | | | **Months 2 and 3** | | | **Months 4, 5 and 6** | | | **Follow Up**[1] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | | **Baseline / D1** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day** | **-42 to -1** | **-40 to -1**[2] | **1** | **8**[3] | **15** | **22**[3] | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window** | | | | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| Vital signs (BP, HR, RR, T)[7] | X | | X | X | X | X | X | X | X | X | X | X | X |
| 12-lead ECG[8] | X | | X | X | | X | | | | | | X | X |
| DXA Scan[9] | X | | | | | | | | | | | X[10] | |
| Laboratory Evaluations[11] | | | | | | | | | | | | | |
| HBsAg, HCV Ab, HIV1 and 2 Ab | X | | | | | | | | | | | | |
| RBC antibody Screen | X | | | | | | | | | | | | |
| Hematology (CBC)[12] | X | X[13] | X | X | X | X | X | X | X | X | X | X | X |
| Haptoglobin[14] | | | X | | | X | | | X | | | X | X |
| EPO levels[15] | | | X | | | X | | | X | | | X | X |
| G6PD screen | X | | | | | | | | | | | | |
| Hepcidin | | | X | | | X | | | X | | | X | X |
| Serum Chemistry[16] | X | | X | X | X | X | X | X | X | X | X | X | X |
| Iron Panel[17] | | | X | | | | | | X | | | X | |
| Carboxyhemoglobin (COHb)[18] | | | X | | | X | X | X | X | X | X | X | |
| ETCO[19] | X | | X | X | X | X | X | X | X | X | X | X | X |
| Coagulation Studies[20] | X | | X | | X | | | | X | | | X | X |

| **Timing** | **Pre-Treatment** | | **Month 1** | | | | **Months 2 and 3** | | | **Months 4, 5 and 6** | | | **Follow Up**[1] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | | **Baseline / D1** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day** | **-42 to -1** | **-40 to -1**[2] | **1** | **8**[3] | **15** | **22**[3] | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window** | | | | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| Urinalysis[21] | X | | X | | X | | | | X | | | X | X |
| Serum or Urine Pregnancy[22] | X | | X | | | | | | | | | | |
| Lipids[23] | | | X | | | | X | | X | | | X | X |
| Hormonal Testing[24] | X | X[25] | X | | | | | | X | | | X | X |
| Serum osteocalcin-N-mid and CTX[26] | | | X | | | | | | X | | | X | |
| 25-hydroxy Vitamin D2 and D3 | | | X | | | | | | X | | | X | |
| Randomization[27] | X | | | | | | | | | | | | |
| Study Drug Administration | | | X | X | X | X | X | X | X | X | X | X[28] | |
| Dispense Study Drug[29] | | | X | X | X | X | X | X | X | X | X | | |
| PK blood sampling[30] | | | X | | X | X | X | X | X | X | X | X | |
| PD Assessments[30] | | | | | | | | | | | | | |
| 2,3-DPG/ATP | | | X | | X | X | X | X | X | X | X | X | |
| [redacted] | | | | | | | | | | | | | |
| Dispense/Collect Menstrual Cycle Diary[32] | | | X | | | | X | | X | X | X | X | X |

| **Timing** | **Pre-Treatment** | | **Month 1** | | | | **Months 2 and 3** | | | **Months 4, 5 and 6** | | | **Follow Up**[1] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | | **Baseline / D1** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day** | **-42 to -1** | **-40 to -1**[2] | **1** | **8**[3] | **15** | **22**[3] | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window** | | | | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| Adverse Events[33] | | | Continuous | | | | | | | | | | X |
| Transfusion Record[34] | X | | X | X | X | X | X | X | X | X | X | X | X |
| Concomitant Medications/Procedures | X | | X | X | X | X | X | X | X | X | X | X | X |
| Rollover to Extension Period[35] | | | | | | | | | | | | X | |

Abbreviations: Ab = antibody; ATP = adenosine triphosphate; BP = blood pressure; CBC= complete blood count; COHb = carboxyhemoglobin; CTX = C-terminal telopeptide; D = day; DPG = diphosphoglycerate; DXA = Dual-energy x-ray absorptiometry; ECG = electrocardiogram; EPO = erythropoietin; ETCO = end tidal carbon monoxide; FSH = follicle stimulating hormone; G6PD = glucose-6-phosphate-dehydrogenase; HBsAg = hepatitis B surface antigen; HCV Ab = hepatitis C virus antibody; HDL-C = high-density lipoprotein-cholesterol; HIV = human immunodeficiency virus; HR = heart rate; PD = pharmacodynamic; PK = pharmacokinetic; PK deficiency = pyruvate kinase deficiency; PKR = pyruvate kinase isoform R; RR = resting rate; W = week.

*Whenever more than one assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (e.g., VS, ECG, blood draw). The timing of these assessments should allow the blood draw to occur at the exact nominal time. The order of procedures may be revised with prior discussion between Sponsor and site.*

[1] The Week 28 Follow-up Visit will only be conducted for patients who do not enter the Extension Period.

[2] To be performed at least 2 days after the first Screening Visit.

[3] In-clinic visits on Day 8 and Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis; in these instances PK/PD sampling would not be required and dispensing of study medication would not be performed. For the Day 8 visit performed by the patient's primary care physician, the primary care medical office will collect a blood sample for hematology using the blood sample collection and shipping supplies from the kit prepared by the designated central laboratory. The kit will be sent to the primary care physician's office. No other testing or procedures will be asked of the primary care physician on Day 8 [VS and serum chemistry will not be required]. The 12-lead ECG scheduled for Day 8 will instead be performed at the main study center on Day 15. For the Day 22 visit performed by the patient's primary care physician, the primary care medical office will collect a blood sample for hematology using the blood sample collection and shipping supplies from the kit prepared by the designated central laboratory. The kit will be sent to the primary care physician's office. No other testing or procedures will be asked of the primary care physician on Day 22. [VS, serum chemistry, coagulation, haptoglobin, EPO level, carboxyhemoglobin, and PK/PD samples will not be required.] The 12-lead electrocardiogram scheduled for Day 22 will instead be performed at the main study center on Day 43 (Week 6). For patients having their Day 8 and/or Day 22 visits performed by the primary care physician, the Principal Investigator will him or herself, or have a qualified research nurse or other designated

site staff member, make telephone contact with the patient to inquire about any adverse events. These must be recorded as if the patient appeared in the main study center. The Principal Investigator will exercise prudent clinical judgment in determining any clinical course of action to take based on any adverse events discovered. These telephone contacts on Day 8 and Day 22 must be explained to the patient in advance and scheduled in advance to maximize the likelihood of successfully making contact.

[4] May be performed either by a designated central laboratory or any participating investigative site's local hematology laboratory.

[5] Medical history, including all relevant prior medical history, current medical conditions, and hematology profile (CBCs) over prior 6 months, will be obtained at the Screening assessment.

[6] A complete physical examination (including neurological examination; genital and rectal examinations will be performed at the discretion of the Investigator) will be obtained at Screening, Baseline, Week 12, and the Follow up Visit (Week 28), or Week 24 for patients rolling over to the Extension Period. The neurological examination must include an assessment of general orientation and mental status including level of alertness (assess as normal or abnormal and specify any abnormality). Limited focused physical examinations will be performed at all other specified visits. Height to be collected at Screening only.

[7] Vital signs will include systolic and diastolic blood pressure, heart rate, respiratory rate, and temperature.

[8] 12-lead ECGs are to be conducted after 5 minutes of recumbency.

[9] If a DXA scan of the hip and spine with T and Z scores has been performed within 3 months preceding the first day of dosing, this may be used to meet this requirement.

[10] Week 24 DXA scan may be performed anytime between Weeks 24 and 28 and must be performed at the same imaging center on the same DXA machine as the original Screening DXA scan.

[11] Laboratory evaluations (hematology, serum chemistry, coagulation studies, and urinalysis) are to be collected in the morning. These should be collected following an overnight fast on Baseline Day 1 Week 6 (Day 43), Week 12 (Day 85), Week 24 (Day 169), and Follow-up Week 28 (Day 197), when the lipid samples are also included.

[12] Three Screening/Baseline samples will be collected for complete blood count (CBC). Samples will be collected in the AM on 3 different days; the samples collected on Baseline/Day 1 may comprise one of these; samples may be taken at the same time as Screening/Baseline hormone assessments (any 2 time points during Screening at least 2 days apart). CBC will include HCT, Hb, red blood cell (RBC) count, absolute reticulocyte count, percent reticulocyte count, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count with automated (or manual, as indicated) differential, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and platelet count. In the event that the designated central laboratory for the study is unable to provide a valid result for any specific component of the defined CBC for a specific patient, the site may be asked to have the test performed at their local laboratory. The result of the local CBC will be entered into the study database along with the local normal reference range.

[13] The second Screening hematology (CBC) should be drawn in the morning (does not have to be fasting), and may be drawn at the same time the patient returns for the second estradiol and free and total testosterone sample.

[14] Haptoglobin will be performed prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

[15] Erythropoietin (EPO) levels will be performed prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

[16] Alkaline phosphatase, sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide (CO2) or bicarbonate, albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and indirect bilirubin, (and estimated creatinine clearance or glomerular filtration rate for screening only, as appropriate).

[17] Iron, total iron-binding capacity (TIBC), transferrin saturation, and ferritin will be performed prior to dosing on Day 1, at the end of Week 12 and at the end of Week 24.

[18] To be collected before the AG-348 morning dose is administered.

[19] End tidal carbon monoxide (ETCO) assessment by CoSense® End Tidal Carbon Monoxide Monitor to be performed only at a subset of investigative sites that elect to participate in this assessment and only during the Core Period. The screening ETCO may be performed at either Screening 1 or Screening 2. ETCO measurement should performed before administration of AG-348 on Baseline/Day 1 and on W2, W3, W6, W9 W12, W16, W20, W24, and W28 (W28 only if not going into Extension Period).

[20] Fibrinogen, activated partial thromboplastin time (aPTT), and international normalized ratio (INR) will be performed at Screening, prior to dosing on Day 1, at the end of Week 2, the end of Week 12, the end of Week 24, and the end of Week 28.

[21] Color, appearance, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, and occult blood. Microscopic inspection of sediment should only be performed for cause or to investigate an abnormal dipstick finding per the Investigator's discretion. Urinalysis will be performed at Screening, prior to dosing on Day 1, at the end of Week 2, the end of Week 12, the end of Week 24, and the end of Week 28.

[22] Must be repeated at any point throughout the study period if pregnancy is clinically suspected.

[23] Total cholesterol, HDL-C, and triglyceride samples will be collected in the morning following an overnight fast.

[24] Three Screening/Baseline samples will be collected for estradiol and testosterone (total and free). Samples will be collected in the AM on 3 different days; the samples collected on Baseline/Day 1 may comprise one of these; the Screening samples may be collected at any 2 time points during Screening at least 2 days apart. Serum estrone, estradiol, and free and total testosterone will then follow the schedule indicated on Day 1 and Weeks 12, 24, and 28. FSH will only be performed at Screening for female patients only for confirmation of post-menopausal status.

[25] The second Screening hormone testing will consist of estradiol and testosterone (free and total) only. Samples should be drawn in the AM (does not need to be fasting), and may be drawn at the same time the patient returns for the second CBC sample.

[26] Serum osteocalcin-N-mid and CTX will be drawn in the AM each time, approximately between 8-10 AM, and after an overnight fast of 10-12 hours.

[27] Randomization will be performed following PKR genotyping and prior to and as close as feasible to dosing on Day 1.

[28] Study drug administration is not required on W24/D169 for patients not continuing into the Extension Period.

[29] Study drug will be dispensed on a 28-day schedule, or on an alternate schedule (< 28 days) as needed to accommodate patient visit schedule and dose modifications. The amount of study drug dispensed should be sufficient to provide an adequate reserve supply of AG-348 to ensure uninterrupted dosing in the event of an unexpected delay for the next scheduled study visit (7 extra days of dosing supply is recommended during the Core Period).

[30] For the first 10 patients treated, extensive PK/PD sampling will be conducted on Days 1 and 15 (see Appendix 15.1, Table 7 for details), followed by limited PK/PD sampling from Week 3 to Week 24 (see Appendix 15.1, Table 8 for details). Limited PK/PD sampling will be conducted on the remainder of patients treated (see Appendix 15.1, Table 8). See Section 10.6, Section 10.7, and Section 10.9 for details on blood sampling for PK and PD assessments, respectively, and guidelines on sample processing and storage.

[redacted]

[32] Menstruating female patients will record their menstrual cycles (start, stop, characteristics) monthly. Paper-based menstrual cycle diaries will be dispensed at study visits approximately every month. The previous month's diary will be collected at these visits as well.

[33] All randomized patients will be evaluated for AEs from the time they sign informed consent until they complete all scheduled study follow-up visits or withdraw consent, whichever occurs first.

[34] All transfusions must be recorded in the eCRF

[35] Patient must have completed 24 weeks of treatment and tolerated AG-348 (may have had dose modifications). Investigator and Medical Monitor or Responsible Medical Officer must agree with the patient continuing on treatment and patient must sign a separate ICF for the Extension Period.

**Table 6: Schedule of Assessments: Extension Period**

| Visit | Month 9 | Month 12 | Month 15 | Month 18 | Month 21 | Month 24 | Month 27 | Month 30 | Follow-up |
|---|---|---|---|---|---|---|---|---|---|
| **Approximate Study Day** | **259** | **349** | **439** | **529** | **619** | **709** | **799** | **889** | **919** |
| **Visit Window** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** |
| Physical Examination/Weight[1] | X | X | X | X | X | X | X | X | X |
| Performance Status | X | X | X | X | X | X | X | X | X |
| Vital signs (BP, HR, RR, T)[2] | X | X | X | X | X | X | X | X | X |
| 12-lead ECG[3] | | X | | X | | X | | X | X |
| DXA Scan | | | | X | | | | X | |
| Laboratory Evaluations[4] | | | | | | | | | |
| Hematology (CBC)[5] | X | X | X | X | X | X | X | X | X |
| Haptoglobin | | X | | X | | X | | X | X |
| EPO levels[6] | | X | | X | | X | | X | X |
| Hepcidin | | X | | X | | X | | X | |
| Serum Chemistry[7] | X | X | X | X | X | X | X | X | X |
| Iron Panel[8] | | X | | X | | X | | X | |
| Carboxyhemoglobin (COHb)[9] | | X | | X | | X | | X | |
| Coagulation Studies[10] | X | X | X | X | X | X | X | X | X |
| Urinalysis[11] | X | X | X | X | X | X | X | X | X |
| Serum or Urine Pregnancy[12] | | | | | | | | | |
| Lipids[13] | X | X | X | X | X | X | X | X | X |
| Hormonal Testing[14] | X | X | X | X | X | X | X | X | X |
| Serum osteocalcin-N-mid and CTX[15] | | | | X | | | | X | |

| Visit | Month 9 | Month 12 | Month 15 | Month 18 | Month 21 | Month 24 | Month 27 | Month 30 | Follow-up |
|---|---|---|---|---|---|---|---|---|---|
| **Approximate Study Day** | **259** | **349** | **439** | **529** | **619** | **709** | **799** | **889** | **919** |
| **Visit Window** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** |
| Study Drug Administration | X | X | X | X | X | X | X | X | |
| Dispense Study Drug[16] | X | X | X | X | X | X | X | | |
| PK blood sampling[17] | X | X | X | X | X | X | X | X | |
| PD Assessments[18] (2,3-DPG/ATP, [redacted]) | X | X | X | X | X | X | X | X | |
| Dispense/Collect Menstrual Cycle Diary[19] | X | X | X | X | X | X | X | X | X |
| Adverse Events[20] | Continuous | | | | | | | | X |
| Transfusion Record[21] | X | X | X | X | X | X | X | X | X |
| Concomitant Medications/Procedures | X | X | X | X | X | X | X | X | X |

Abbreviations: ATP = adenosine triphosphate; BP = blood pressure; CBC= complete blood count; COHb = carboxyhemoglobin; CTX = C-terminal telopeptide; DPG = diphosphoglycerate; DXA = Dual-energy x-ray absorptiometry; ECG = electrocardiogram; EPO = erythropoietin; HDL-C = high-density lipoprotein-cholesterol; HIV = human immunodeficiency virus; HR = heart rate; PD = pharmacodynamic; PK = pharmacokinetic; PK deficiency = pyruvate kinase deficiency; PKR = pyruvate kinase isoform R; RR = resting rate; W = week.

*Whenever more than one assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (e.g., VS, ECG, blood draw). The timing of these assessments should allow the blood draw to occur at the exact nominal time. The order of procedures may be revised with prior discussion between Sponsor and site.*

[1] A focused physical examination (including neurological examination; genital and rectal examinations will be performed at the discretion of the Investigator) will be obtained every 3 months; a complete physical examination will be performed at the Month 30 visit. The neurological examination must include an assessment of general orientation and mental status including level of alertness (assess as normal or abnormal and specify any abnormality).

[2] Vital signs will include systolic and diastolic blood pressure, heart rate, respiratory rate, and temperature

[3] 12-lead ECGs are to be conducted after 5 minutes of recumbency.

[4] Laboratory evaluations (hematology, serum chemistry, coagulation studies, and urinalysis) are to be collected in the morning. These should be collected following an overnight fast.

[5] CBC will include HCT, Hb, red blood cell (RBC) count, absolute reticulocyte count, percent reticulocyte count, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count with automated (or manual, as indicated) differential, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and platelet count. In the event that the designated central laboratory for the study is unable to provide a valid result for any specific component of the defined CBC for a specific patient, the site may be asked

to have the test performed at their local laboratory. The result of the local CBC will be entered into the study database along with the local normal reference range.

[6] Erythropoietin (EPO) levels will be performed prior to dosing at Month 12, Month 18, Month 24, and Month 30.

[7] Alkaline phosphatase, sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide (CO2) or bicarbonate, albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and indirect bilirubin.

[8] Iron, total iron-binding capacity (TIBC), transferrin saturation, and ferritin will be performed prior to dosing at Month 12, Month 18, Month 24, and Month 30.

[9] To be collected before the AG-348 morning dose is administered.

[10] Fibrinogen, activated partial thromboplastin time (aPTT), and international normalized ratio (INR) will be performed at each study visit.

[11] Color, appearance, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, and occult blood. Microscopic inspection of sediment should only be performed for cause or to investigate an abnormal dipstick finding per the Investigator's discretion. Urinalysis will be performed prior to dosing at each study visit.

[12] Must be obtained at any point throughout the study period if pregnancy is clinically suspected. Effective contraception must be continued throughout the Extension Period in all patients and any pregnancies must be reported (see Section 11.3).

[13] Total cholesterol, HDL-C, and triglyceride samples will be collected in the morning following an overnight fast.

[14] Serum estrone, estradiol, and free and total testosterone.

[15] Serum osteocalcin-N-mid and CTX will be drawn in the AM each time, approximately between 8-10 AM, and after an overnight fast of 10-12 hours.

[16] Study drug will be dispensed on a 3-month schedule, or on an alternate schedule (< 3 months) as needed to accommodate patient visit schedule and dose modifications. The amount of study drug dispensed should be sufficient to provide an adequate reserve supply of AG-348 to ensure uninterrupted dosing in the event of an unexpected delay for the next scheduled study visit (14 extra days of dosing supply is recommended during the Extension Period).

[17] Predose; PK sampling will only include AG-348 and AGI-8702 concentrations.

[18] Predose.

[19] Menstruating female patients will record their menstrual cycles (start, stop, characteristics) monthly. Paper-based menstrual cycle diaries will be dispensed and collected at each study visit.

[20] All randomized patients will be evaluated for AEs from the time they sign informed consent until they complete all scheduled study follow-up visits or withdraw consent, whichever occurs first.

[21] All transfusions must be recorded in the eCRF.

**Table 7: Schedule of Assessments: Extensive PK/PD Sampling during the Core Period**

| Sample Timing/Interval | Month 1 | | | | | | | | Months 2 and 3 | | | Months 4, 5 and 6 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Visit | Baseline / D1<br>W2 / D15 | | | | | | | W3 | W6 | W9 | W12 | W16 | W20 | W24 |
| Study Day | 1/15 | | | | | | | 22 | 43 | 64 | 85 | 113 | 141 | 169 |
| Visit Window | ± 2 D (D15) | | | | | | | ± 2 D | ± 7 D | ± 7 D | ± 7 D | ± 7 D | ± 7 D | ± 7 D |
| Timing | Pre-dose[1] | 30 min[2] | 1 hr[2] | 2 hr[2] | 4 hr[3] | 8 hr[3] | 12 hr[3] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] |
| PK blood sample | X | X | X | X | X | X | X[4] | X | X | X | X | X | X | X |
| 2,3 DPG/ATP | X | X | X | X | X | X | X[4] | X | X | X | X | X | X | X |
| [redacted] | | | | | | | | | | | | | | |

Abbreviations: ATP = adenosine triphosphate; D = day; DPG = diphosphoglycerate; PD = pharmacodynamic; PK = pharmacokinetic; PKR = pyruvate kinase isoform R; W = week.

[1] The acceptable time window will be within 60 minutes prior to study treatment dose administration for the pre-dose PK/PD sample. Study drug administration is not required on W24/D169 for patients not continuing into the Extension Period.

[2] The acceptable time window will be within ± 5 minutes of the scheduled collection time for the 30 minute, 1 and 2 hour PK/PD samples.

[3] The acceptable time window will be within ± 30 minutes of the scheduled collection time for the 4, 8, and 12 hour PK/PD samples.

[4] To be collected on Day 1 only.

[redacted]

[6] If the 12 hour time point cannot be collected at site on Day 1, an 8 hour time point may be collected instead.

[redacted]

**Table 8: Schedule of Assessments: Limited PK/PD Sampling during the Core Period**

| Sample Timing/Interval | Month 1 | | | Months 2 and 3 | | | Months 4, 5 and 6 | | |
|---|---|---|---|---|---|---|---|---|---|
| Visit | Baseline / D1 | W2 | W3 | W6 | W9 | W12 | W16 | W20 | W24 |
| Study Day | 1 | 15 | 22 | 43 | 64 | 85 | 113 | 141 | 169 |
| Visit Window | - | ± 2 D | ± 2 D | ± 2 D | ± 7 D | ± 7 D | ± 7 D | ± 7 D | ± 7 D |
| Timing | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] |
| PK blood sample | X | X | X | X | X | X | X | X | X |
| 2,3 DPG/ATP | X | X | X | X | X | X | X | X | X |
| [redacted] | | | | | | | | | |

Abbreviations: ATP = adenosine triphosphate; D = day; DPG = diphosphoglycerate; PD = pharmacodynamic; PK = pharmacokinetic; PKR = pyruvate kinase isoform R; W = week.

[1] The predose blood sample for plasma PK/PD analysis should be collected within 60 minutes prior to study treatment dose administration. Study drug administration is not required on W24/D169 for patients not continuing into the Extension Period.

[redacted]

## 15.2. Eastern Cooperative Oncology Group Performance Status Scoring

| Grade | Symptomatology |
|---|---|
| 0 | Fully active, able to carry on all pre-disease performance without restriction |
| 1 | Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work |
| 2 | Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours |
| 3 | Capable of only limited self-care, confined to bed or chair more than 50% of waking hours |
| 4 | Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair |
| 5 | Dead |

Source: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and Response Criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-655.

## 15.3. National Cancer Institute Common Terminology Criteria for Adverse Events

The NCI CTCAE, Version 4.03, can be accessed using the following link:

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

## 15.4. Potential Drug Interactions

Strong inhibitors of CYP3A4 (listed in Table 9) are not permitted for use with AG-348. Based on modeling of AG-348 metabolism and tolerability data from the Phase 1 MAD study, it is thought that moderate inhibitors of CYP3A4 do not pose a risk to patients dosed with AG-348.

In-vivo data from the Phase 1 MAD show AG-348 induces CYP3A4 and induces its own metabolism, presumably via CYP3A4. The use of strong CYP3A4 inducers in combination with AG-348 is expected to reduce AG-348's efficacy. Therefore, administration of strong CYP3A4 inducers (listed in Table 10) with AG-348 is not permitted. Strong inhibitors of drug transport (listed in Table 11) are not permitted for use with AG-348.

Digoxin is not permitted for use with AG-348.

Induction of CYP3A4 by AG-348 is expected to reduce the efficacy of certain sensitive co-medications. Co-administration of AG-348 with the drugs in Table 12 should be done with caution, as their efficacy may be reduced.

Of note, in accordance with Inclusion Criteria 14, women in the trial utilizing oral contraception must utilize barrier methods while taking AG-348.

The expected patient co-medications deferoxamine, deferasirox, deferiprone, and oral penicillin are not expected to interact with AG-348.

**Table 9: Strong and Moderate CYP3A4 Inhibitors**

| Strong CYP3A4 Inhibitors: Contraindicated | Moderate CYP3A4 Inhibitors: No Action |
|---|---|
| Indinavir | Aprepitant |
| Nelfinavir | Erythromycin[1] |
| Ritonavir | Fluconazole |
| Clarithromycin | Verapamil[1] |
| Itraconazole | Diltiazem[1] |
| Ketoconazole | |
| Nefazodone | |
| Saquinavir | |
| Suboxone | |
| Telithromycin | |
| Grapefruit juice[2] | |

Strong Inhibitor; > 5 fold increase in AUC

Moderate Inhibitor; > 2 fold, < 5 fold increase in AUC

[1] Erythromycin, verapamil and diltiazem are contraindicated because they are strong P-gp inhibitors (see Table 11)

[2] Although classified as a moderate CYP3A4 inhibitor, grapefruit and grapefruit juice are prohibited

**Table 10: Strong CYP3A4 Inducers**

| **Strong CYP3A4 Inducers: Contraindicated** | |
|---|---|
| Efavirenz | Phenytoin |
| Nevirapine | Pioglitazone |
| Carbamazepine | Rifabutin |
| Modafinil | Rifampin |
| Oxcarbazepine | St. John's Wort |
| Phenobarbital | Troglitazone |

Note: Corticosteroids may induce CYP3A4. Although the use of corticosteroids is not prohibited, their use should be minimized as much as medically feasible.

**Table 11: Strong P-glycoprotein Inhibitors**

| **Strong P-gp Inhibitors: Contraindicated** | |
|---|---|
| Amiodarone | Felodipine |
| Azithromycin | Itraconazole |
| Captopril | Ketoconazole |
| Carvedilol | Lopinavir |
| Clarithromycin | Ritonavir |
| Conivaptan | Quercetin |
| Cyclosporine | Quinidine |
| Diltiazem | Ranolazine |
| Dronedarone | Ticagrelor |
| Erythromycin | Verapamil |

**Table 12: Sensitive CYP3A4 Substrates**

| **Sensitive CYP3A4 Substrates: Substitute or Use with Caution** | | | | |
|---|---|---|---|---|
| | **Antihistamines:** | **Miscellaneous:** | | |
| | Chlorpheniramine | Alfentanil | Finasteride | Salmeterol |
| | | Aprepitant | Gleevec | Sildenafil |
| **Benzodiazepines:** | **Calcium Channel Blockers:** | Aripiprazole | Haloperidol | Sirolimus |
| Alprazolam | Amlodipine | Boceprevir | Irinotecan | Sorafenib |
| Diazepam→3OH | Lercanidipine | Buspirone | LAAM | Sunitinib |
| Midazolam | Nifedipine | Cafergot | Lidocaine | Tamoxifen |
| Triazolam | Nisoldipine | Caffeine→TMU | Methadone | Taxol |
| | Nitrendipine | Cilostazol | Nateglinide | Telaprevir |
| **Immune Modulators:** | | Cocaine | Nevirapine | Terfenadine |
| Tacrolimus (FK506) | **HMG CoA Reductase Inhibitors**: | Codeine-N-demethylation | Ondansetron | Torisel |
| | Atorvastatin | Dapsone | Pimozide | Trazodone |
| **Steroid 6beta-OH:** | Cerivastatin | Dextromethorphan | Propranolol | Vemurafenib |
| Estradiol | Lovastatin | Docetaxel | Quetiapine | Vincristine |
| Hydrocortisone (and other glucocorticoids) | Simvastatin | Domperidone | Quinine | Zaleplon |
| Progesterone | | Eplerenone | Risperidone | Ziprasidone |
| Testosterone | | Fentanyl | Romidepsin | Zolpidem |

## 15.5. Declaration of Helsinki

**WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI:**

Recommendations Guiding Medical Doctors in Biomedical Research Involving Human Subjects

Adopted by the 18th World Medical Association (WMA) General Assembly, Helsinki, Finland, June 1964 and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975, 35th WMA General Assembly, Venice, Italy, October 1983, and the 41st WMA General Assembly, Hong Kong, September 1989, the 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996; 52nd WMA General Assembly, Edinburgh, Scotland, October 2000; 53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added); 55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added); and 59th WMA General Assembly, Seoul, October 2008.

**A. INTRODUCTION**

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.
   The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of subjects, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my subject will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the subject's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
8. In medical practice and in medical research, most interventions involve risks and burdens.

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.
10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

**B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH**

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.
12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, Sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.
15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the Sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any SAEs. No change to the protocol may be made without consideration and approval by the committee.
16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on subjects or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects

must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.
25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.
27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.
28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious subjects, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.
30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

## C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the subjects who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:
    - The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
    - Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the subjects who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.
33. At the conclusion of the study, subjects entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.
34. The physician must fully inform the subject which aspects of the care are related to the research. The refusal of a subject to participate in a study or the subject's decision to withdraw from the study must never interfere with the subject-physician relationship.
35. In the treatment of a subject, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the subject or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

# Clinical Study Protocol AG348-C-003
# EudraCT No. 2015-000484-13
# A Phase 2, Open Label, Randomized, Dose Ranging, Safety, Efficacy, Pharmacokinetic and Pharmacodynamic Study of AG-348 in Adult Patients with Pyruvate Kinase Deficiency

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|---|---|
| Study Sponsor: | Agios Pharmaceuticals, Inc.<br>88 Sidney Street<br>Cambridge, MA 02139-4169<br>Phone: 617-649-8600<br>Fax: 617-649-8618 |
| Responsible Medical Officer: | [redacted], MD, PhD<br>[redacted]<br>Agios Pharmaceuticals, Inc.<br>Phone: [redacted]<br>Email: [redacted] |
| Study Medical Monitor | [redacted], MD<br>[redacted]<br>On behalf of Agios Pharmaceuticals, Inc.<br>Mobile Phone: [redacted]<br>Office Phone: [redacted]<br>Email: [redacted] |
| Document Version (Date):<br>Revised | Version 1.0 (05 January 2015)<br>Amendment 1, Protocol Version 2.0 (02 February 2015) Final<br>Amendment 2, Protocol Version 3.0 (05 August 2015) Final<br>Amendment 3, Protocol Version 4.0 (10 November 2015) Final |

This study will be conducted according to the protocol and in compliance with Good Clinical Practice, the ethical principles stated in the Declaration of Helsinki, and other applicable regulatory requirements.

**CONFIDENTIALITY NOTE:**

The information contained in this document is confidential and proprietary to Agios Pharmaceuticals, Inc. Any distribution, copying, or disclosure is strictly prohibited unless such disclosure is required by federal regulations or state law. Persons to whom the information is disclosed must know that it is confidential and that it may not be further disclosed by them.

# INVESTIGATOR'S AGREEMENT

I understand that all documentation provided to me by Agios or its designated representative(s) concerning this study that has not been published previously will be kept in strict confidence. This documentation includes the study protocol, Investigator's Brochure, case report forms, and other scientific data.

This study will not commence without the prior written approval of a properly constituted Institutional Review Board (IRB). No changes will be made to the study protocol without the prior written approval of Agios and the IRB, except where necessary to eliminate an immediate hazard to the patient.

I have read, understood, and agree to conduct this study as outlined in the protocol and in accordance with the guidelines and all applicable government regulations.

| Investigator Name (printed) | Investigator Signature | Date |
|---|---|---|

Investigational site or name of institution and location (printed)

# 2. SYNOPSIS

**Name of Sponsor/Company:**
Agios Pharmaceuticals, Inc.

**Name of Investigational Product:**
AG-348

**Name of Active Ingredient:**
AG-348 sulfate hydrate

**Title of Study:**
A Phase 2, Open Label, Randomized, Dose Ranging, Safety, Efficacy, Pharmacokinetic and Pharmacodynamic Study of AG-348 in Adult Patients with Pyruvate Kinase Deficiency

**Study Center(s):**
This study will be conducted at multiple study centers.

**Phase of Development:** 2

***Objectives:***

**Core Period**

**Primary:**

- Evaluate the safety and tolerability of up to 24 weeks of AG-348 administration in patients with pyruvate kinase deficiency (PK deficiency).

**Secondary:**

- Evaluate the pharmacokinetics (PK) of AG-348 and the metabolite AGI-8702.
- Evaluate the pharmacodynamic (PD) response of adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG) after administration of AG-348.
- Evaluate indicators of clinical activity of AG-348 in patients with PK deficiency, including changes in hemoglobin (Hb), hematocrit (HCT), reticulocyte count, haptoglobin (Hp), carboxyhemoglobin (COHb), lactate dehydrogenase (LDH), total and indirect bilirubin, erythropoietin (EPO), ferritin, and transferrin saturation (serum iron/iron binding capacity).

[redacted]

**Extension Period**

**Primary:**

- Evaluate the safety and tolerability of up to 30 months of AG-348 administration in patients with PK deficiency.

**Secondary:**

- Evaluate the PK of AG-348 and the metabolite AGI-8702.
- Evaluate the PD response of ATP and 2,3-DPG after administration of AG-348.
- Evaluate indicators of clinical activity of AG-348 in patients with PK deficiency, including changes

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in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation (serum iron/iron binding capacity).

[redacted]

**Methodology:**

Study AG348-C-003 is a Phase 2, open label, two arm, multicenter, randomized, dose-ranging study in adult patients with PK deficiency; the study will be divided in to a Core Period and an Extension Period. During the Core Period, patients will receive multiple doses of AG-348 for up to 24 weeks; patients who are eligible can enter the Extension Period to receive AG-348 for up to 2 years following the end of the Core Period. Patients with PK deficiency confirmed by red blood cell (RBC) PK enzymatic assay performed at Screening will be eligible to participate in this study. At Week 25, patients who safely tolerate AG-348 and demonstrate clinical activity of AG-348 may be eligible to immediately roll over to the Extension Period for continued treatment. Patients who complete treatment at the end of the Core Period (24 weeks) will undergo follow-up assessment 4 weeks after the last dose of study drug. If a patient discontinues at any other time (including early discontinuation or discontinuation during the Core or Extension Period), the follow-up assessments will be conducted 4 weeks after discontinuation. Patients with toxicity suspected to be related to study drug will continue follow-up until the adverse event (AE) resolves, is declared chronic by the Investigator, or the patient is lost to follow-up.

For the Core Period, up to 25 patients will be initially randomized on an open-label 1:1 basis to each of two twice-daily (BID) doses of AG-348 (up to 50 patients total; see Figure 1, Study Schema). The dose of Arm 1 is 300 mg of AG-348 administered orally (PO) every 12 hours (q12h, BID). The dose of Arm 2 is 50 mg of AG-348 administered PO q12h (BID). Randomization will be stratified by PKR mutation in order to maintain balance as much as possible across the dose arms for the specific mutations expected to be most frequently enrolled. The PKR mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H) and all other mutations ("other"). Mutation status is defined by the presence of at least one of the indicated mutations; patients with more than one stratified mutation will be assigned based on Sponsor's discretion.

The doses for each arm of the Core Period have been selected from the AG348-C-001 single ascending dose (SAD) and AG348-C-002 multiple ascending dose (MAD) studies in healthy adult volunteers to represent the range of doses/exposures that were safely tolerated and resulted in maximal or near-maximal PD effects on 2,3-DPG and ATP.

Because PK deficiency is a rare disease with a limited eligible patient population and because the underlying pathophysiology and clinical phenotype of affected patients is heterogeneous due to the wide variety of mutations in PKR that cause the disease, it is important to focus closely on dose findings in this first-in-patient study. Therefore, in addition to initiating this study with 2 different doses of AG-348 administered q12h, a Data Review Team (DRT) will be established to review study data on a regular basis and adapt the study design, dose and schedule of AG-348.

The DRT will monitor safety on an on-going basis and meet at regular intervals of approximately every

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6 weeks, or *ad hoc*, as necessary, for as long as any patients are still in the Core Period to review AEs, vital signs (VS), clinical laboratory assessments (hematology, clinical chemistry, coagulation, and urinalysis), and electrocardiograms (ECGs). The DRT will also review available PK/PD data and indicators of clinical activity (e.g., changes from baseline in Hb). These DRT meetings will also include data review for all patients that may be under treatment in the Extension Period. If there are no patients still being treated in the Core Period, and the only patients being treated are those in the Extension Period, then the frequency of the DRT meetings will reduce to approximately every 3 months in order to match the frequency of patient visits (and new data collection) in the Extension Period. The DRT will be comprised of the study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer.

Beginning 6 weeks after the first patient is dosed in the Core Period or *ad hoc* as necessary, and proceeding according to the schedule indicated above (approximately every 6 weeks during the Core Period, and approximately every 3 months during the Extension Period once all patients have completed the Core Period), the DRT will review cumulative safety data, available PK/PD data, and clinical activity data. Based on the DRT's recurring reviews, the DRT may exercise one or more of the following options during the Core Period:

- Continue treatment and enrollment in existing arms without change.
- Add 1 new dose arm (Arm 3) to enroll up to 25 patients at a dose to be determined; the dose for Arm 3 may be lower or higher than Arm 1 and Arm 2 doses, but will not exceed 360 mg q12h; and the dose regimen may be less frequent than q12h.
- Terminate or suspend enrollment to allow further review of clinical data in Arm 1 and/or Arm 2 (and/or potential Arm 3). Enrollment in an arm could be terminated or suspended to allow further review, for example, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity.
- Re-assign patients' doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the patients in the terminated arm will remain in their original arm, i.e., they will not count towards the enrollment quota of the arm whose dose and schedule is being adopted.
- Implement specific genotype restrictions to enrollment in one or more arms to ensure representation of patients with genotypes of greatest clinical relevance.

The DRT may exercise one or more of the following options during the Extension Period:

- Continue treatment without change;
- Re-assign patients' doses and schedule to an existing dose and schedule that has been determined to be safer, and/or have a better PD response, or produce signs of clinical activity;
- Terminate or suspend treatment to allow further review of clinical data (eg, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity).

The data that the DRT will review to make these decisions is expected to include, but are not necessarily limited to, the following:

- *Safety Observations:* all AEs; VS, clinical laboratory (hematology, clinical chemistry,

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coagulation, and urinalysis), and ECG;

- *PK and PD Observations:* including changes in 2,3-DPG and ATP;
- *Indicators of Clinical Activity:* including changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, EPO, total and indirect bilirubin, ferritin, and transferrin saturation.

If a third dose arm is implemented, the dose of AG-348 selected will not exceed 360 mg BID, as this was the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy volunteers. The PK/PD sampling schedule in a potential third dosing arm will be determined by Sponsor's discretion and may follow either the extensive or limited PK/PD sampling schedules as specified.

The DRT will monitor the data in an ongoing manner as described and may make a decision to terminate enrollment in an arm if unacceptable AEs are observed, PD activity is limited, and/or markers of clinical activity indicate no effect. Unacceptable AEs are defined as ≥ Grade 3 AEs (using National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAEv4.03]) that are assessed as at least possibly related to AG-348, with the exception of Grade 3 headache, hot flash/flushing, nausea, vomiting, and/or diarrhea that is transient in duration (< 24 hours) or able to be medically managed to ≤ Grade 2 within 24 hours. The DRT may also exercise discretion to terminate enrollment in an arm if it observes a frequency of Grade 2 AEs that would make it difficult to support long-term dosing.

Patients in the Extension Period will continue on the dose they were randomized to in the Core Period, unless the DRT had reason to establish a different dose/schedule during the course of the Core Period (the DRT will not propose a dose higher than 360 mg q12h).

Due to the potential for AG-348-mediated aromatase inhibition, dual-energy x-ray absorptiometry (DXA) scan (hip and spine) will be performed at Screening (if patient has not had prior DXA scan within 3 months of Day 1) to obtain T and Z scores. These data are intended to serve as a baseline measure of bone mineral density for all enrolling patients, and are deemed of particular importance for those who enter the longer term Extension Period after completing 24 weeks of treatment (Core Period). All patients will have a second DXA scan in the interval between Weeks 24 and 28 for the Core Period. Patients in the Extension Period will have additional DXA scans at Months 18 and 30.

As the number of enrolling arms changes in the study (for example, from 2 to 3), the randomization scheme will adjust to enable balanced randomization into each actively accruing arm. Randomization and stratification will cease in the event that only a single arm is left enrolling.

Depending on possible early termination of 1 or both of the initial 2 arms, or the addition of a third arm, the study could enroll up to a maximum of 75 patients.

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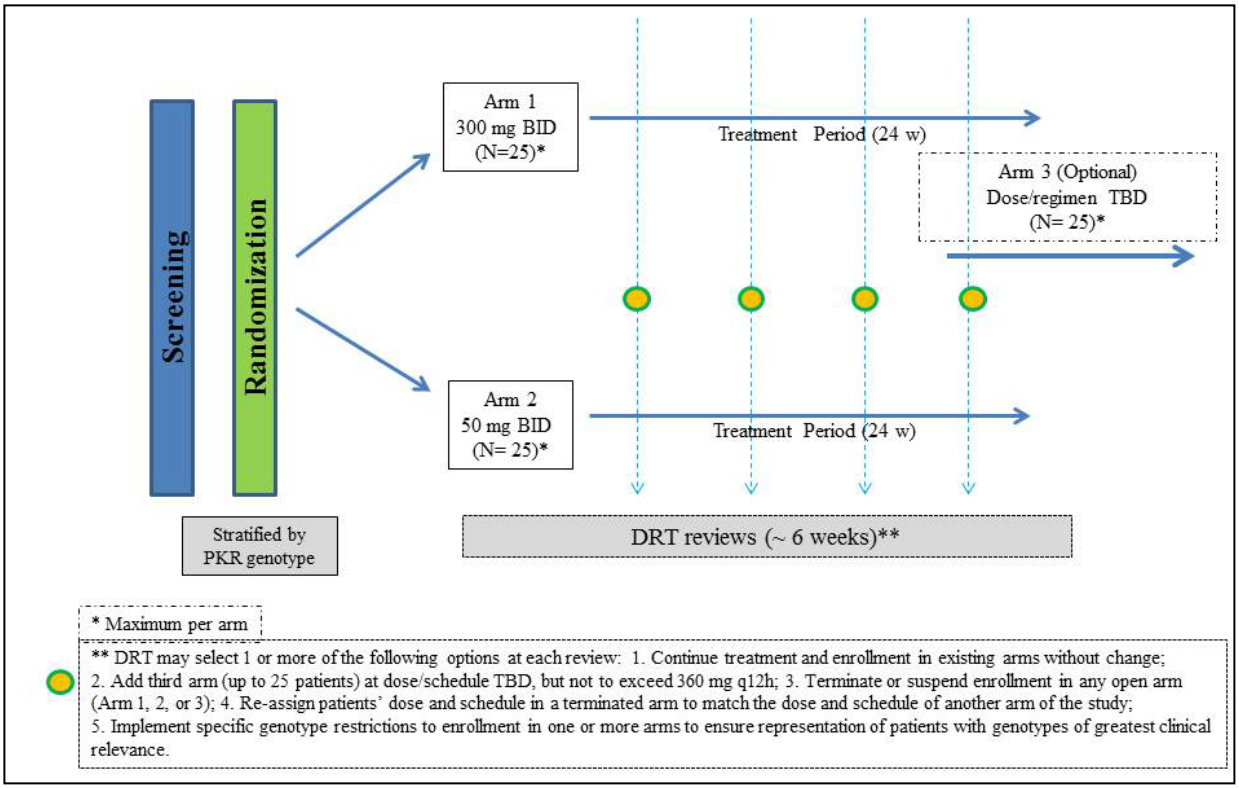
**Figure 1: Study Schema: Core Period**




Abbreviations: BID (q12h) = twice-daily (every 12 hours); DRT = data review team; PKR = pyruvate kinase red blood cell isoform; TBD= to be determined; w = week(s).

***Visit Schedule***

Screening assessments will occur within 28 days prior to the first dose of study treatment. During the Core Treatment Period, patients will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12), and monthly through Week 24 (Weeks 16, 20, and 24). Patients who safely tolerate AG-348 of AG-348 through Week 24 (Core Period) may be eligible to immediately enter the Extension Period for continued treatment for up to 2 years after completion of the Core Period upon agreement of the treating Investigator and the Medical Monitor or Responsible Medical Officer. Study visits for safety and clinical activity assessments will occur approximately every 3 months during the Extension Period. All patients will undergo a follow-up assessment 4 weeks after the last dose of AG-348, regardless of whether this was due to early discontinuation, the last dose in the Core Period for a patient who chooses not to continue in the Extension Period, or the last dose of the Extension Period.

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**Dose Modifications for Safety and/or Increase in Hb Level**
The Investigator will monitor all patients for safety and tolerability. Modification of an individual patient's dose of AG-348 will be based on AEs and/or observed changes in Hb level as described in Section 9.7.1 and Section 9.7.2. The same criteria for dose modifications will apply in the Extension Period as in the Core Period.

**Number of patients (planned):** Up to approximately 75 patients.

**Diagnosis and main criteria for inclusion:**

**Inclusion criteria:**

For entry into the Core Period, patients must meet all of the following criteria during the Screening or other specified period:

1. Signed written informed consent obtained prior to performing any study procedure, including screening procedures.
2. Male or female, aged 18 years and older.
3. Known medical history of PK deficiency.
4. All patients must have documented clinical laboratory confirmation of PK deficiency by RBC pyruvate kinase enzymatic assay performed at Screening by a designated central laboratory. Patients with prior documentation of PK deficiency by RBC enzymatic assay will have a reconfirmation of this result during Screening as a condition of enrollment.
    i. In the event that a patient's screening pyruvate kinase enzymatic assay is negative (i.e., shows normal pyruvate kinase activity), the patient will be eligible for enrollment if the genotyping shows a mutant genotype that has been previously documented in the literature to be associated with PK deficiency. If the genotyping shows a previously undescribed mutation in the PKR gene, then the eligibility for enrollment will be determined on a case-by-case basis by the Coordinating Investigator and Medical Monitor in discussion with the Investigator. If no mutation is defined, then the patient will not be eligible.
5. All patients must have genotypic characterization of the mutant PKR gene performed by the designated central laboratory at Screening.
6. All patients must have genotypic characterization of the UGT1A1 gene performed by a designated central laboratory to document whether they may have underlying Gilbert's Disease. Patients with Gilbert's Disease are eligible to enroll.
7. Males must have Hb $\leq$ 12.0 g/dL; females must have Hb $\leq$ 11.0 g/dL.
8. All patients must be considered transfusion independent as defined by: no greater than 3 units of RBCs transfused in the 12-month period up to the first day of study dosing and no transfusions

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within 4 months of first day of study dosing. Patients who have received more transfusion support than described above will be evaluated for eligibility on a case-by-case basis by the Medical Monitor.

9. Eligible patients may still have their spleens in place, or may have undergone prior splenectomy. For splenectomized patients:
   i. Must have undergone their procedure at least 6 months prior to Screening.
   ii. Must be current in their vaccinations for Pneumococcal Conjugate (PCV13), Pneumococcal Polysaccharide (PPSV23), Quadrivalent Meningococcal vaccine, and *Haemophilus influenzae* type b as recommended by Centers for Disease Control and Prevention's (CDC) Advisory Committee on Immunization Practices (ACIP) or immunization advisory groups in Canada and the European Union (for patients enrolled in Canada and the EU). [http://www.cdc.gov/vaccines/schedules/downloads/adult/adult-schedule.pdf] [Any missing vaccinations may be administered during the Screening period. If the patient requires both PCV13 and PPSV23, PCV13 must be given before PPSV23, if possible. Administration of PPSV23 should follow PCV13 by at least 8 weeks; it is permissible to give PCV13 during Screening followed by PPSV23 following the initiation of AG-348 treatment.]
10. Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 2. (Appendix 15.2)
11. Patients must be taking at least 1 mg of folic acid daily for at least 21 days prior to first dose and continue daily during study participation.
12. Adequate organ function, defined as:
   i. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤ 1.5 × upper limit of normal (ULN) (unless the increased AST is assessed by the Investigator as due to hemolysis).
   ii. Normal or elevated levels of serum bilirubin. In patients with serum bilirubin > ULN, the elevation must be attributed to hemolysis with or without Gilbert's syndrome and must not be choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease.
   iii. Serum creatinine ≤ 1.25 × ULN. If serum creatinine > 1.25 × ULN, then 24-hour measured or calculated (Cockcroft-Gault) glomerular filtration rate (GFR) ≥ 60 mL/min.
   iv. Absolute neutrophil count (ANC) > $1.0 \times 10^9$/L.
   v. Platelet count ≥ $100 \times 10^9$/L.
   vi. Activated partial thromboplastin time (aPTT) and international normalized ratio (INR) ≤ 1.25 × ULN, unless the patient is receiving therapeutic anticoagulants.
13. Women of childbearing potential (WOCBP) must agree to abstain from sexual intercourse or to use an acceptable/effective method of contraception (i.e., condom plus spermicide, condom plus oral contraceptive, condom plus intrauterine device [IUD], condom plus diaphragm with spermicide) from as soon as feasible during the Screening period until 30 days following the last

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dose of AG-348. Abstinence is an acceptable method only when this is in line with the normal life style of the patient, meaning that the patient plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence, e.g., calendar, sympathothermal and post-ovulation methods, and withdrawal are not acceptable methods of contraception.

i. WOCBP include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, and/or bilateral oophorectomy) or is not post-menopausal. Post-menopausal is defined as:
ii. Amenorrhea ≥ 12 consecutive months without another cause, and a documented serum follicle stimulating hormone (FSH) level > 35 mIU/mL;
iii. Amenorrhea ≥ 12 consecutive months in women ≥ 62 years old (FSH testing is not required).

14. WOCBP must have a negative serum or urine pregnancy test within 72 hours before start of AG-348 dosing.
15. Women must not be breastfeeding.
16. Male patients, with the exception of those who have undergone vasectomy at least 6 months prior to Screening, must agree to abstain from sexual intercourse or, if sexually active, to use a condom with spermicide as contraception (regardless of their female partner's childbearing potential or their partner's use of their own contraception) from Day 1 of dosing until 30 days following the last dose of AG-348. Abstinence is an acceptable method only when this is in line with the normal life style of the patient, meaning that the patient plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence, e.g., selective timing of intercourse based on partner's calendar, sympathothermal and post-ovulation methods, and withdrawal are not acceptable methods of contraception.

For entry into the Extension Period, patients must meet the following criteria:

17. Signed written informed consent obtained prior to performing any study procedure during the Extension Period.
18. Patient must have completed 24 weeks of treatment during the Core Period and tolerated AG-348 (defined as having completed 24 weeks with or without permitted dose modifications)
19. The patient's treating Investigator agrees that there is a potential for clinical benefit to continued treatment and recommends participation in the Extension Period
20. The Sponsor's designated Medical Monitor or Responsible Medical Officer approves the patient's participation in the Extension Period
21. As applicable, the patient must agree to continue to follow the same sexual

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abstinence/contraception rules as stated in Inclusion Criteria 13 and 16.

**Exclusion criteria:**

Patients who meet any of the following criteria at Screening or prior to dosing on Day 1 will not be enrolled in the Core Period:

1. Hemoglobin level > 12.0 g/dL if male; Hb > 11.0 g/dL if female.
2. Additional diagnosis of any other congenital or acquired blood disorder, including glucose-6-phosphate-dehydrogenase (G6PD) deficiency, or any other hemolytic anemia process except for mild allo-immunization as a consequence of transfusion therapy.
3. Iron overload (hemosiderosis or concurrent hemochromatosis) sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac, hepatic, or pancreatic insufficiency.
4. Prior bone marrow or stem cell transplant.
5. Clinically symptomatic cholelithiasis or cholecystitis. (Prior cholecystectomy is not exclusionary. Patients with symptomatic cholelithiasis or cholecystitis may be rescreened once the disorder has been treated and clinical symptoms have resolved.)
6. Currently enrolled in another therapeutic clinical trial involving on-going therapy with any investigational or marketed product or placebo. Concurrent participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480) is permitted.
7. Exposure to any investigational drug, device, or procedure within 28 days prior to Screening.
8. Concurrent medical condition that could compromise participation in the study such as:
    a. Poorly controlled hypertension (defined as systolic blood pressure (BP) > 150 mm Hg or diastolic BP > 90 mm Hg) refractory to medical management.
    b. History of recent (within < 6 months from Screening date) congestive heart failure; myocardial infarction or unstable angina pectoris; or hemorrhagic, embolic, or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.
    c. Currently active infection requiring the use of parenteral anti-microbial agents or that is greater than Grade 3 (CTCAEv4.03) within 6 months of first dose.
    d. A pattern or frequency of post-splenectomy sepsis that in the assessment of the Investigator could reasonably be expected to interfere with the ability of the patient to complete the 24 week study participation.
    e. Positive test for hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV) antibody with signs of active Hepatitis B or C virus infection.

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f. Positive test for human immunodeficiency virus (HIV) 1 or 2 antibody.

g. Diabetes mellitus judged to be in poor control by the Investigator or requiring > 3 anti-diabetic agents counting insulin (all insulins are considered one agent); use of insulin per se is not exclusionary.

h. History of any primary malignancy with the exception of: curatively treated non-melanomatous skin cancer; curatively treated cervical or breast carcinoma in situ; or other primary tumor treated with curative intent and no known active disease present and no treatment administered during the last 3 years.

9. Undergone major surgery within 6 months of first dose.

10. Current or recent history of psychiatric disorder that in the opinion of the Investigator or Medical Monitor could compromise the ability of the patient to cooperate with study visits and procedures.

11. Use of any of the restricted list of products known to strongly inhibit cytochrome P450 (CYP) 3A4 drug metabolism (Appendix 15.4, Table 9) within 5 days prior to Day 1 dosing; or to strongly induce CYP3A4 metabolism (Appendix 15.4, Table 10) within 28 days prior to Day 1 dosing; or to strongly inhibit P-glycoprotein (P-gp) transporter (Appendix 15.4, Table 11) within 5 days prior to Day 1 dosing; or digoxin within 5 days prior to Day 1 dosing. For patients who require chronic inhaled glucocorticoid therapy, Investigators should confer with the Medical Monitor for additional guidance.

12. Serum bilirubin > ULN attributable to factors other than hemolysis and/or Gilbert's syndrome.

13. Male patients with heart-rate corrected QT interval -Fridericia's method (QTcF) interval > 450 msec, or female patients with QTcF interval > 470 msec with the exception of patients with a left bundle branch block (LBBB). Medical Monitor approval needed in patients with a LBBB.

14. Cardiac dysrhythmias judged as clinically significant by the Investigator or requiring therapy with drugs that are primarily substrates of CYP3A4.

15. History of allergy to sulfonamides if characterized by acute hemolytic anemia, anaphylaxis, or rash of erythema multiforme type or Stevens-Johnson syndrome.

16. Any other medical or psychological condition deemed by the Investigator to be likely to interfere with a patient's ability to understand and sign informed consent; cooperate with study visits, tests, and procedures; or otherwise safely and reliably participate in the study.

Patients will not be permitted to enter the Extension Period if:

17. The patient experienced AEs during the Core Period that are considered by the treating

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Investigator or the Sponsor's designated Medical Monitor or Responsible Medical Officer to pose a significant safety risk to the patient if treatment were to be extended.

**Investigational product, dosage, and mode of administration:**
AG-348 sulfate hydrate capsules will be provided as 5 mg, 25 mg, or 100 mg (free-base equivalent) of AG-348 sulfate hydrate without excipients in dark green opaque (5 mg), Swedish orange (25 mg), or white opaque (100 mg) gelatin capsules (size 2 capsules for all dose strengths). AG-348 will be administered PO BID. The number of capsules per dose will vary by assigned dose group. AG-348 will be administered with water and may be administered with or without food.

**Reference therapy, dosage and mode of administration:**
Not applicable.

**Duration of treatment:**
The duration of treatment for all patients in the Core Period will be up to 24 weeks. Patients who safely tolerate AG-348 and for whom the Investigator agrees with continuation of AG-348 treatment may be eligible to immediately roll over to the Extension Period for continued treatment (up to 2 years following completion of the Core Period).

**Criteria for evaluation:**

**Safety:**

Monitoring of AEs in randomized patients, including determination of serious AEs (SAEs) and AEs leading to discontinuation; safety laboratory parameters (hematology, chemistry, urinalysis, coagulation); physical examination findings (including neurological examination); VS; 12-lead ECGs, and DXA scans. Adverse events will be graded using CTCAE, Version 4.03. Serum sex hormone levels (testosterone [total and free], estrone, and estradiol), bone turnover markers (serum osteocalcin-N-mid and serum C-terminal telopeptide [CTX]), 25-hydroxy vitamin D2 and D3, total cholesterol, high-density lipoprotein-cholesterol (HDL-C), and triglycerides will be monitored for evidence of potential inhibition of aromatase by AG-348. Menstruating female patients will also keep a paper-based menstrual cycle diary throughout the study.

**Indicators of Clinical Activity:**

Monitoring of potential indicators of clinical activity will include evaluating changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation.

**Pharmacokinetics:**

Approximately the first 10 patients treated in the Core Period, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 15.1, Table 7. The remainder of treated patients will undergo limited PK sampling as detailed in Appendix 15.1, Table 8. During the Core Period, serial blood sampling for determination of concentration-time profiles of AG-348 and its metabolite AGI-8702 will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of AG-348 and AGI-8702 will be obtained. During the Extension Period, predose PK samples will be drawn for the measurement of trough levels of AG-348 and AGI-8702 at each study visit (every 3 months; see Appendix 15.1, Table 6). AG-348 and AGI-8702 will be analyzed using qualified assays

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to determine concentrations in plasma. Pharmacokinetic parameters on Day 1 and Day 15 will be computed using standard non-compartmental methods based on observed plasma AG-348 and AGI-8702 concentrations.

**Pharmacodynamics:**

Pharmacodynamic assessments during the Core Period will include 2,3-DPG, ATP (secondary objectives), [redacted]
Approximately the first 10 patients treated during the Core Period will undergo extensive PD sampling as detailed in Appendix 15.1, Table 7. The remainder of treated patients will undergo limited PD sampling as detailed in Appendix 15.1, Table 8. During the Core Period, serial blood sampling for determination of levels of ATP and, 2,3-DPG will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of ATP and 2,3-DPG will be obtained. During the Extension Period, predose PD samples will be drawn for the measurement of trough levels of 2,3-DPG, ATP, [redacted] at each study visit (every 3 months; see Appendix 15.1, Table 6). Adenosine triphosphate and 2,3 DPG will be analyzed using qualified assays to determine concentrations in whole blood. Pharmacodynamic parameters on Day 1 and Day 15 will be computed based on observed whole blood ATP and 2,3-DPG concentrations.

[redacted]

**Statistical methods:**

The primary objective during the Core Period of this Phase 2 study is to evaluate the safety and tolerability of up to 24 weeks of AG-348 in patients with PK deficiency. Therefore, analyses will be primarily descriptive in nature; no formal hypothesis testing will be conducted. All analyses will be conducted separately within each dose arm, or pooled when appropriate Analyses of safety and of indicators of clinical activity will be conducted for the Core Period, Extension Period, and overall, if applicable. For the Core Period, the data to be analyzed will include all collected data through 24 weeks of treatment for patients who directly enter the Extension period. For patients who do not enter the Extension Period, the analyses will include all collected data through the duration of treatment (24 weeks or less) plus 4 week follow up data. For patients who move directly from the Core to the Extension Period, the 4 week follow up data will be analyzed as part of the Extension Period.

Summaries will be produced for disposition, baseline disease characteristics and demographic data including genotype, safety measurements, PK, PD parameters and indicators of clinical activity. Data

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**Name of Investigational Product:**
AG-348

**Name of Active Ingredient:**
AG-348 sulfate hydrate

from each AG-348 dose group will be analyzed separately, and pooled across all dose groups where appropriate. All data will also be listed by individual patient. Categorical variables will be summarized by frequency distributions (number and percentages) and continuous variables will be summarized by descriptive statistics (mean, standard deviation [SD], median, minimum, and maximum).

Populations for analysis will include a Safety Analysis Set, a PK Analysis Set, and an Efficacy Analysis Set. The Safety Analysis set will include all patients who are enrolled and receive any dose of study treatment; this population will be the primary set for the analysis of safety data and the default analysis set for all data analyses unless otherwise stated. Patients will be classified according to treatment received, where treatment received is defined as the assigned treatment if it is received at least once, or as the first treatment received if assigned treatment is never received. The PK Analysis Set will include all patients in the Safety Analysis Set with sufficient plasma sample data to assess PK parameters. Results of the potential PD activity of AG-348 will also be based on the PK analysis set. The Efficacy Analysis Set will include all patients who are enrolled and achieve at least 50% compliance with their assigned dose intensity for at least 4 weeks of continued dosing. Additional efficacy analyses may be performed on subsets of patients with other degrees of compliance with their assigned dose intensity and/or duration of dosing. The Efficacy Analysis Set will be the primary set for the analysis of preliminary clinical activity data. Patients will be classified according to assigned treatment. [redacted]

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and the incidence of treatment-emergent AEs (TEAEs) (new or worsening from baseline) will be summarized by primary system organ class (SOC), preferred term, CTCAE Version 4.03 severity, outcome, action taken with study drug, and relationship to study drug by dose group. Separate summaries will be produced for all TEAEs, treatment-related AEs (those considered by the Investigator as at least possibly drug related), SAEs, discontinuations due to AEs, and AEs ≥ Grade 3 severity. Individual patient listings will be provided for any deaths, SAEs, AEs leading to interruption and/or reduction of study drug dose, and AEs leading to discontinuation of treatment. Analyses of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined as any AE with onset beginning at the day of first administration of study drug, throughout the treatment period until 30 days after the last dose of study drug, or any event that was present at baseline but worsened in intensity or was subsequently considered drug-related by the Investigator through the end of the study.

Descriptive statistics will be provided for clinical laboratory values (e.g., hematology, serum chemistry, coagulation studies, urinalysis) and VS data, presented as both actual values and changes from baseline relative to each on-study evaluation. Shift analyses will be conducted for selected laboratory parameters based on the baseline CTCAE grade to maximum CTCAE grade. Where applicable CTCAE terms do not exist, a grading system based on the upper and/or lower limits of normal will be used to classify the results.

Electrocardiogram analyses will include individual patient listings and summaries of abnormal and clinically significant ECG results. Actual values and changes from baseline in the portion of the ECG wave from the beginning of the P wave to the beginning of the QRS complex (PR), QRS, heart rate-

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corrected QT intervals (QTc) will be summarized by visit and dose arm.

Data collected from the menstrual diaries such as the start and stop dates of the menses and the patient reported characteristics of the menses will be presented in a by-patient listing. Additional descriptions of the data may also be performed.

Concomitant medications will be coded using the WHO Drug Dictionary. Concomitant medications prior to and after the start of the study drug will be listed by patient, and summarized by ATC term and dose arm.

Descriptive statistics will be used to summarize PK parameters for the parent compound AG-348 and the metabolite AGI-8702 for each dose group, and where appropriate, for the entire population. Pharmacokinetic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation.

Descriptive statistics will be used to summarize PD parameters for 2,3-DPG and ATP for each dose group, and where appropriate for the entire population. Pharmacodynamic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation %.

Analyses of serum sex hormones will use appropriate graphic displays and statistical analyses to evaluate patient change from baseline for each parameter. These analyses will include summaries of actual values and change from baseline using appropriate descriptive statistics (mean, SD, median, min and max) as described above.

Analyses evaluating indicators of potential clinical activity of AG-348 in patients with PK deficiency will include changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation (serum iron/iron binding capacity). Characterization of Hb response (e.g., % of patients whose Hb increases by a certain amount), as well as time to Hb response, and duration of Hb response will be explored, among others.

The study database will be locked and statistical analysis will be performed after all patients have completed the Core Period. The results of this analysis will be presented in a clinical study report (CSR). Additional data collected during the Extension Period after the CSR database lock will be analyzed for inclusion in a subsequent CSR addendum.

**Interim Review**

No formal statistical analysis will be conducted. Safety data will be reviewed on an ongoing basis by the DRT, who will meet to review safety, PK, PD, and preliminary clinical activity data at regular intervals (approximately every 6 weeks during the Core Period and approximately every 3 months during the Extension Period once all patients have completed the Core Period) throughout the duration of the study. The composition of the DRT, its meeting schedule, the data to be reviewed, and the decisions it is empowered to make have been described previously.

Additional interim reviews of data may be conducted to support decision making concerning the current clinical study, the sponsor’s development programs in general, or in case of any safety concerns.

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| **Name of Sponsor/Company:**<br>Agios Pharmaceuticals, Inc. |
| **Name of Investigational Product:**<br>AG-348 |
| **Name of Active Ingredient:**<br>AG-348 sulfate hydrate |
| **Sample Size**<br>Due to the rare disease setting, the minimal sample size may be determined by feasibility. Depending on possible early termination of 1 or both of the initial 2 arms or the addition of a 3rd dose arm, the study could enroll up to a maximum of 75 patients. |

# 3. TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

## TABLE OF CONTENTS

## LIST OF TABLES



## LIST OF FIGURES

# 4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

| Abbreviation or Specialist Term | Explanation |
|---|---|
| 2,3-DPG | 2,3-diphosphoglycerate |
| ACIP | Advisory Committee on Immunization Practices |
| ADP | Adenosine diphosphate |
| AE | Adverse event |
| ALC | Absolute lymphocyte count |
| ALP | Alkaline phosphatase |
| ALT | Alanine aminotransferase |
| ANC | Absolute neutrophil count |
| aPTT | Activated partial thromboplastin time |
| AST | Aspartate aminotransferase |
| ATP | Adenosine triphosphate |
| AUC | Area under the curve |
| $AUC_{0\text{-}12hr}$ | Area under the plasma concentration versus time curve from 0 to 12 hours |
| $AUC_{0\ 24hr}$ | Area under the plasma concentration versus time curve from 0 to 24 hours |
| $AUC_{0\text{-}\infty}$ | Area under the plasma concentration versus time curve from 0 to infinity |
| BCRP | Breast cancer resistance protein |
| BID | Twice daily |
| BP | Blood pressure |
| BUN | Blood urea nitrogen |
| CBC | Complete blood count |
| CDC | Centers for Disease Control and Prevention |
| $C_{max}$ | Maximum plasma concentration |
| $CO_2$ | Carbon dioxide |
| COHb | Carboxyhemoglobin |
| CSR | Clinical study report |
| CTCAE | Common Terminology Criteria for Adverse Events |
| CTX | Serum C-terminal telopeptide |
| CV | Cardiovascular |

| Abbreviation or Specialist Term | Explanation |
|---|---|
| DDI | Drug-drug interaction |
| CYP | Cytochrome P450 |
| DILI | Drug-induced liver injury |
| DLT | Dose-limiting toxicity |
| DRT | Data review team |
| DXA | Dual-energy x-ray absorptiometry |
| ECG | Electrocardiogram |
| ECOG | Eastern Cooperative Oncology Group |
| eCRF | Electronic case report form |
| EDC | Electronic Data Capture |
| EPO | Erythropoietin |
| FDA | Food and Drug Administration |
| FSH | Follicle stimulating hormone |
| G6PD | Glucose-6-phosphate-dehydrogenase |
| GCP | Good Clinical Practice |
| GFR | Glomerular filtration rate |
| GLP | Good Laboratory Practice |
| Hb | Hemoglobin |
| HBsAg | Hepatitis B surface antigen |
| HCT | Hematocrit |
| HCV | Hepatitis C virus |
| HDL-C | High-density lipoprotein-C |
| HIV | Human immunodeficiency virus |
| Hp | Haptoglobin |
| $IC_{50}$ | Concentration of drug that achieved half-maximal inhibition |
| ICH | International Conference on Harmonization |
| IEC | Independent Ethics Committee |
| INR | International normalized ratio |
| IRB | Institutional Review Board |
| IUD | Intrauterine device |
| LBBB | Left bundle branch block |
| LDH | Lactate dehydrogenase |

| Abbreviation or Specialist Term | Explanation |
|---|---|
| MAD | Multiple ascending dose |
| MCH | Mean corpuscular hemoglobin |
| MCHC | Mean corpuscular hemoglobin concentration |
| MCV | Mean corpuscular volume |
| MedDRA | Medical Dictionary for Regulatory Activities |
| mPKR | PKR mutants |
| MTD | Maximum tolerated dose |
| NCI | National Cancer Institute |
| NOEL | No-observed-effect-level |
| NOAEL | No-observed-adverse-effect-level |
| P-gp | P-glycoprotein |
| PCV13 | Pneumococcal Conjugate |
| PD | Pharmacodynamic |
| PEP | Phosphoenolpyruvate |
| PK | Pharmacokinetic |
| PK deficiency | Pyruvate kinase deficiency |
| PKM2 | Pyruvate kinase isoform M2 |
| PKR | Pyruvate kinase isoform R |
| PO | Oral(ly) |
| PPSV23 | Pneumococcal polysaccharide |
| PR | The portion of the ECG wave from the beginning of the P wave to the beginning of the QRS complex |
| q12h | Every 12 hours |
| q24h | Every 24 hours |
| QD | Once-daily |
| QTc | Heart-rate corrected QT interval |
| QTcB | Corrected QT interval - Bazett correction formula |
| QTcF | Corrected QT interval - Fridericia's method |
| RBC | Red blood cell (count) |
| RDW | Red cell distribution width |
| SAD | Single ascending dose |
| SAE | Serious adverse event |
| SAP | Statistical analysis plan |

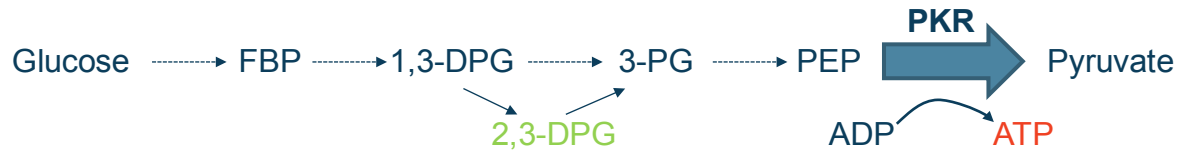
| Abbreviation or Specialist Term | Explanation |
|---|---|
| SD | Standard deviation |
| SOC | System organ class |
| $t_{½}$ | Apparent terminal half-life |
| TIBC | Total iron-binding capacity |
| $T_{max}$ | Time to maximum plasma concentration |
| TEAE | Treatment-emergent adverse event |
| ULN | Upper limit of normal |
| VS | Vital signs |
| $V_{ss}$ | Volume of distribution at steady-state |
| Vz/F | Mean apparent volume of distribution |
| WBC | White blood cell |
| WMA | World Medical Association |
| WOCBP | Women of childbearing potential |
| WT | Wild type |

# 5. INTRODUCTION

## 5.1. Pyruvate Kinase Deficiency

Pyruvate kinase deficiency (PK deficiency) is a glycolytic enzymopathy that results in non-spherocytic hemolytic anemia with a variable clinical presentation, ranging from mild or fully compensated forms to life-threatening neonatal anemia and life-long chronic hemolytic anemia associated with severe, debilitating co-morbidities. Pyruvate kinase deficiency is caused by mutations in the PKLR gene, which results in defective pyruvate kinase isoform R (PKR) in the red blood cell (RBC). As shown in the diagram below, PKR catalyzes the final and irreversible step in glycolysis, converting phosphoenolpyruvate (PEP) to pyruvate, and formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP). Mature RBCs rely almost exclusively on the process of glycolysis to generate the energy carrier molecule ATP. PKR is thus a key enzyme for maintaining energy homeostasis in erythrocytes, and it has been proposed that ATP levels are critical for optimally maintaining RBC membrane integrity (van Wijk and van Solinge 2005).

**Figure 2: Red Cell Glycolysis**




Abbreviations: 1,3 DPG = 1,3 diphosphoglycerate; 2,3 DPG = 2,3 diphosphoglycerate; 3-PG = 3-phosphoglycerate; ADP = adenosine diphosphate; ATP = adenosine triphosphate; FBP = fructose 1,6-bisphosphate; PEP = phosphoenolpyruvate; PKR = red blood cell-specific form of pyruvate kinase
Note: Not all steps in glycolysis are shown.

The majority of mutations in PKR that have been described have a deleterious effect on PKR catalytic activity, protein stability, and/or protein expression. Pyruvate kinase deficiency is associated with reduced RBC survival as well as impaired red cell maturation. Mature erythrocytes lack mitochondria relying predominantly on glycolysis to generate ATP. It is hypothesized that insufficient energy production promotes erythrocyte hemolysis due to impaired maintenance of cellular membrane homeostasis.

Pyruvate kinase deficiency is an autosomal recessive disease, in which both homozygotes and compound heterozygotes develop hemolytic anemia. More than 200 different mutations in the PKLR gene have been identified in patients with PK deficiency to date, and the majority of these mutations are single nucleotide missense mutations (Zanella, et al. 2005). The prevalence of PK deficiency, based on the most common pyruvate kinase mutation in Southern Europe, is estimated at 51 cases per million in the Caucasian population (Beutler and Gelbart 2000).

Some patients with PK deficiency have a clinically severe disease course that can present as a severe hemolytic anemia in early infancy, requiring immediate care which typically includes blood transfusion support (Christensen, et al. 2011; Ghidini, et al. 1991; Hennekam, et al. 1990). Infants with PK deficiency may have erythroblastosis fetalis with hepatosplenomegaly, extramedullary hematopoiesis, and severe indirect hyperbilirubinemia, which is associated with a

high risk of kernicterus. Hyperbilirubinemia associated with the hemolytic state often requires supportive treatment with intensive phototherapy. For other infants, the hyperbilirubinemia caused by hemolysis can require exchange transfusion, as well as supportive transfusion to keep their hematocrit (HCT) levels >60%.

Adults with PK deficiency are reported to have life-long hemolysis and the subsequent associated co-morbidities including anemia and transfusion dependence. Other co-morbidities include frequent miscarriages, aplastic crises, as well as symptoms associated with an acute on chronic hemolytic anemia (Rider, et al. 2011). Since unconjugated bilirubin is often chronically elevated, pigmented gallstones are common in children and adults. Severe and sometimes life-threatening iron deposition occurs and is typically progressive.

The current therapeutic options for patients with PK deficiency are supportive. Most require life-long treatment, including blood transfusions at a frequency depending on the disease state. Long-term surveillance for systemic iron overload, even in transfusion-independent patients, is standard as is the use of chelation therapy. Case reports of cure by allogeneic bone marrow transplant have been published (Tanphaichitr, et al. 2000) but have been infrequently performed. Splenectomy, while not curative, can reduce the need for supportive blood transfusions and decrease bilirubin levels in some patients. However, this intervention is associated with significant morbidity including infection risk, and possible increased risk of thrombosis.

## 5.2. AG-348

AG-348 is an orally available, allosteric activator of PKR. It is hypothesized that drug intervention with AG-348 restores glycolytic pathway activity and normalizes red cell metabolism *in vivo*. Biochemical experiments demonstrate that AG-348 is a potent, pan-activator of many PKR alleles associated with PK deficiency. Pyruvate kinsase deficiency red cells and their progenitors are characterized by changes in metabolism associated with defective glycolysis, including a build-up of PEP and 2,3-diphosphoglycerate (2,3-DPG), and lowered ATP levels. Treatment of PK deficiency patient red cells *ex vivo* with AG-348 results in increased ATP levels, and reductions in PEP and 2,3-DPG, consistent with pharmacological activation of PKR enzyme activity. *In vitro* studies against wild type (WT) PKR enzymes and *ex vivo* studies in blood from mice, monkeys and humans indicate that AG-348 activates WT PKR with a similar potency and efficacy as it does with the mutant versions. *In vivo* studies in mice confirm the potency of AG-348 in increasing WT PKR enzyme activity and modulating the levels of downstream markers such as ATP and 2,3-DPG. This therapeutic approach may be an effective way to correct the underlying pathology of PK deficiency and, importantly, provide clinical benefit to patients.

### 5.2.1. Summary of Nonclinical Data

A series of exploratory pharmacology studies were conducted to characterize the ability of AG-348 to activate WT PKR and anemia-associated PKR mutants (mPKR) *in vitro*, *ex vivo*, and *in vivo*. Additional details regarding nonclinical data can be found in the AG-348 Investigator's Brochure

#### 5.2.1.1. Pharmacology

AG-348 is a potent, broad-spectrum activator of PKR with low nM potency against both WT and mutant enzymes. AG-348 is also able to effectively stabilize mPKR enzymes that are hypersensitive to thermal denaturation, suggesting that AG-348 may be able to increase PKR pathway activity by preventing destabilization and degradation of mPKR enzymes. Its major metabolite, AGI-8702, has low potency mixed activity against PKR and mutant isoforms and can increase the thermostability of some mPKR, albeit with significantly weaker potency than that of AG-348.

The effect of AG-348 and AGI-8702 on PKR activity and a number of downstream pathway markers was evaluated in human RBCs. AG-348 dose-response curves in human whole blood showed potent activation of PKR, while AGI-8702 is a weak activator of the PKR enzyme. In whole blood from mice treated with AG-348, $^{13}$C-glucose labeling through the PKR reaction was increased by 80% demonstrating AG-348-mediated activation of the WT PKR enzyme. AG-348 treatment of RBCs from healthy human donors potently increased levels of the PKR reaction product ATP consistent with activation of PKR.

The effects of AG-348 on PKR activity and RBC metabolism also were assessed in blood samples from patients with PK deficiency. AG-348 activated PKR and induced metabolic changes (increased ATP levels and decreased 2,3-DPG levels) consistent with increased glycolytic pathway activity in RBCs from PK deficiency patients with different mutations in the PKR enzyme.

Assessments of binding inhibition potential and enzymatic activity were evaluated in a panel of 89 receptors, ion channels, and enzymes. Although AG-348 is a histamine H3 antagonist/inverse agonist and an aromatase inhibitor, no effects of histamine H3 modulation have been observed in safety pharmacology or toxicology studies. Effects consistent with aromatase inhibition were seen in toxicology studies (Section 5.2.1.4).

#### 5.2.1.2. Safety Pharmacology

Safety pharmacology studies demonstrated that neither AG-348 nor AGI-8702 exhibited significant inhibition of the human ether-à-go-go related gene (hERG) current (concentration of drug that achieved half-maximal inhibition [$IC_{50}$] 29.4 µM and > 10 µM, respectively). Additionally, in the monkey cardiovascular (CV) study, no CV effects were seen at any dose level. No effects were seen in AG-348 Good Laboratory Practice (GLP)-compliant Irwin and respiratory safety pharmacology studies in Sprague-Dawley rats. AG-348 administered via oral gavage had a dose-dependent emetic activity in the ferret at ≥ 30 mg/kg.

#### 5.2.1.3. Pharmacokinetics

Absorption, distribution, metabolism, and excretion (ADME) studies of AG-348 were performed in Sprague-Dawley rats, beagle dogs, and cynomolgus monkeys. Both oral (PO) and intravenous (IV) routes were evaluated at doses ranging from 1 to 50 mg/kg. A dose of 200 mg (50 mg/kg) was administered PO in the food-effect evaluation in monkeys. The PK of AG-348 in animal species is characterized by rapid oral absorption, medium to high total body plasma clearance (CLp), and high volume of distribution at steady-state ($V_{ss}$) in rats, dogs, and monkeys.

AG-348 has a medium to long apparent terminal half-life ($t_{1/2}$) in the rat, dog, and monkey. Medium to high oral bioavailability was observed for AG-348 in animals. A high-fat diet led to lower oral absorption of AG-348 in monkeys.

AG-348 showed low brain penetration in rats (brain/plasma ratio of 5.7% to 9.1%) based on area under the plasma concentration versus time curve from 0 to 24 hours ($AUC_{0\ 24hr}$) following single and repeated-dose administration, respectively. Metabolism appears to be the major elimination pathway for AG-348 with minimal biliary or urinary excretion.

Both *in vitro* and *in vivo*, the predominant metabolite was the N-dealkylated product, AGI-8702. There were no metabolites unique to human liver microsomes and no direct Phase 2 metabolites. The plasma metabolite profile of AG-348 did not change with repeat or ascending dosing, suggesting that AG-348 is not susceptible to metabolic switching with increasing or prolonged dose administration.

AG-348 is a weak direct inhibitor of cytochrome P450 (CYP) 2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 (testosterone 6β-hydroxylation). There was no direct inhibition of CYP1A2, CYP2B6, or CYP3A4/5 (midazolam 1′-hydroxylation) by AG-348. There was evidence of metabolism-dependent inhibition of CYP2C19 (largely reversible) and CYP3A4 (largely irreversible) by AG-348. AG-348 is considered a weak time-dependent CYP3A4 inhibitor. AGI-8702 is a weak direct inhibitor of CYP2C9 and CYP2C19. AG-348 has the potential to cause an induction-related drug-drug interaction (DDI) with sensitive CYP2B6 and CYP3A4 substrates.

The routes of metabolism for AG-348 are via multiple CYPs with CYP3A4 contributing > 70% of the total. CYP1A2, CYP2C9, and CYP2C8 contribute approximately 6%, 10%, and 7% to the remaining metabolism of AG-348; other isoforms contribute < 4% each.

AG-348 is a substrate for P-glycoprotein (P-gp), but not breast cancer resistance protein (BCRP). AG-348 is an inhibitor of P-gp (91% and 99% inhibition at 41 and 411 μM, respectively), but does not inhibit BCRP. AGI-8702 is not an inhibitor of P-gp or BCRP under the concentrations tested (5 and 100 μM).

#### 5.2.1.4. Toxicology

In rats, the no-observed-effect-level/no-observed-adverse-effect-level (NOEL/NOAEL), determined as 2000 mg/kg, was associated with area under the plasma concentration versus time curve from 0 to 12 hours ($AUC_{0\text{-}12hr}$) values 223- to 526-fold the projected human efficacious $AUC_{0\text{-}12hr}$ value. In dogs, clinical observations consistent with anaphylactoid reactions were seen, and the maximum tolerated dose (MTD) was 62.5 mg/kg, which was associated with an $AUC_{0\text{-}12hr}$ value 5.7-fold the projected efficacious $AUC_{0\text{-}12hr}$ value. The NOEL/NOAEL in dogs was 10 mg/kg, which was associated with an $AUC_{0\text{-}12hr}$ value 0.8-fold the projected efficacious $AUC_{0\text{-}12hr}$ value. In monkeys, the NOAEL was 1000 mg/kg, with only non-adverse emesis and body weight loss seen; this dose was associated with an $AUC_{0\text{-}12hr}$ value 70-fold the projected efficacious $AUC_{0\ 12hr}$ value. Based on the results of single-dose studies, the rat and the monkey were chosen as the most appropriate species for further evaluation in toxicology studies.

Dose-limiting toxicity (DLT) in cynomolgus monkeys was defined in non-GLP 5-day and 14-day repeat-dose studies as emesis, inappetence, and weight loss. These toxicities became dose limiting at $AUC_{0\text{-}12hr}$ values 27- to 34-fold the projected efficacious $AUC_{0\text{-}12hr}$ value, and

precluded meaningful evaluation of other toxicities at this exposure level. Potential other effects at this exposure level were observed in a number hematology and serum chemistry parameters as well as in lymphoid organs. Additionally, minimal potential effects in kidneys (renal tubulointerstitial nephritis) and heart (myocardial degeneration), which could not be differentiated from spontaneous background lesions, were seen when monkeys were exposed to AG-348 $AUC_{0\text{-}12hr}$ values ≥ 27- to 34-fold the projected human efficacious $AUC_{0\text{-}12hr}$ value.

In GLP-compliant 28-day monkey study, the dose of 150 mg/kg/day (75 mg/kg/dose twice daily [BID]) was the NOAEL. Effects were limited to increased liver weights without serum chemistry or microscopic correlate. At this dosage level, the Day 27 $AUC_{0\text{-}12hr}$ values were 8.9- and 8.5-fold the projected efficacious $AUC_{0\text{-}12hr}$ value in males and females respectively. In the same study, the low dosage of 20 mg/kg/day (10 mg/kg/dose) resulted in $AUC_{0\text{-}12hr}$ values that approximated the efficacious $AUC_{0\text{-}12hr}$ value, and there were no test article-related effects seen. The next highest dose of 50 mg/kg/day (25 mg/kg/dose) was the NOEL and was associated with $AUC_{0\text{-}12hr}$ values 3.1- and 2.6-fold the projected efficacious $AUC_{0\text{-}12hr}$ value in males and females respectively.

In the GLP-compliant 28-day rat study, the NOAEL in males was the highest dose tested, 600 mg/kg/day (300 mg/kg/dose), and the NOAEL in females was the lowest dose tested, 20 mg/kg/day (10 mg/kg/dose). At the 600 mg/kg/day dosage level in males, AG-348-related findings were limited to mild effects on hematology, serum chemistry, and urinalysis parameters, and microscopic findings in the adrenal gland (minimal to mild vacuolation of the adrenal zona glomerulosa and decreased thickness of the zona fasciculate), liver (minimal to mild hepatocellular hypertrophy), kidney (minimal tubular vacuolation), pancreas (minimal to moderate decreased zymogen granules), heart (minimal myocardial vacuolation), and prostate (minimal to mild decreased secretion). All findings were fully reversible over the 14-day recovery period with the exception of decreased serum glucose levels and decreased prostate secretion. In females, the highest dosage tested was 200 mg/kg/day (100 mg/kg/dose); adverse effects observed were similar to those observed in the 600 mg/kg/day males, with the exception that in females, fewer effects in hematology and serum chemistry parameters were seen, and also in females, adverse effects in the reproductive organs consistent with aromatase inhibition were observed.

In the 13-week repeat-dose rat study, adverse effects in males were identified in the testes and consisted of seminiferous tubular degeneration, spermatid retention, and Leydig cell hypertrophy. These effects were defined as adverse at dose levels at which they are expected to impair fertility. These effects were either not present or present with incidence and severity similar to that of the vehicle group in lower dose levels. Adverse effects in females included uterine atrophy and increased folding of the luminal surface; these effects were defined as adverse at the dose level at which they are expected to impair fertility

In the 13-week repeat-dose study in monkeys, no adverse effects were identified, and no new effects were identified when compared to the 4-week repeat-dose study. Similar to what occurred on the 4-week study, inappetence and emesis during the initial 1-2 weeks of dosing occurred, precluding evaluation of higher doses.

AG-348 inhibits human aromatase activity with an $IC_{50}$ of 2050 nM (based on human placental microsomes) and rat aromatase with an $IC_{50}$ of 493 nM (based on rat ovarian microsomes). In the GLP-compliant 28-day rat study, histologic effects consistent with aromatase inhibition were

seen in the female reproductive tract at the mid- and high-dosage levels (100 and 200 mg/kg/day) and included incomplete corpora lutea; ovarian follicular cysts; ovarian cystic, luteinized follicles; uterine atrophy; vaginal mucification; and altered cyclicity. Although these findings were minimal to mild and were fully reversible (over 14 days), they were considered adverse and the next lower dosage evaluated, 20 mg/kg/day (10 mg/kg/dose BID) was the NOAEL in females. The Day 27 $AUC_{0\text{-}12hr}$ value associated with this dosage level was 6.9-fold the projected human efficacious $AUC_{0\text{-}12hr}$ value. The potential for aromatase inhibition effects occurring in female rats at $AUC_{0\text{-}12hr}$ values > 6.9-fold and < 53-fold the projected efficacious $AUC_{0\text{-}12hr}$ value has been addressed in a 13-week rat study. In this study using doses between the NOAEL and LOAEL in the 28-day study, the NOAEL for histologic lesions of the uterus that may be associated with aromatase inhibition resulted in an $AUC_{0\text{-}12hr}$ that was 26-fold the projected efficacious value. Notably, due to the potency difference of AG-348 against rat versus human aromatase inhibition, there is potential for a 4-fold wider margin for aromatase inhibition in humans versus rats. AGI-8702 is not an aromatase inhibitor.

### 5.2.2. Summary of Clinical Data

To date, 72 healthy adult volunteers have been exposed to AG-348 in 2 clinical studies, a single ascending dose (SAD) study and a multiple ascending dose (MAD) study, with 31 of these subjects exposed to a single AG-348 dose under fasted conditions; 5 exposed to 2 doses of AG-348 under fasted and fed conditions; and 36 exposed to repeated doses of AG-348 for up to 14 days[1]. The following discussion of clinical data refers only to healthy adult volunteer subjects, as this is the first clinical trial in which patients with PK deficiency will be treated with AG-348.

#### 5.2.2.1. Pharmacokinetics

The pharmacokinetics (PK) of AG-348 showed low to moderate variability between subjects. After single doses of AG-348 from 30 to 2500 mg, AG-348 was rapidly absorbed as indicated by the short time to maximum plasma concentration ($T_{max}$). There appeared to be some lengthening of the period of absorption at higher doses as indicated by higher $T_{max}$ and a less than proportional increase in maximum plasma concentration ($C_{max}$). Dose-normalized area under the curve (AUC) generally remained constant over the dose range studied, suggesting that AG-348 total exposure increased in a dose-proportional manner. The mean $t_{1/2}$ ranged from 17.8 to 20.4 hours when samples were collected through 72 hours and from 50.3 to 79.3 when samples were collected through 120 hours. However, this terminal elimination phase contributed little to overall exposure of AG-348, as indicated by the small difference between $AUC_{0\text{-}12}$ and area under the plasma concentration versus time curve from 0 to infinity ($AUC_{0\text{-}\infty}$), suggesting a shorter effective half-life of approximately 3 to 6 hours. AG-348 was extensively distributed (mean apparent volume of distribution [$V_z/F$] range of 271 to 1148 L) and had a moderate rate of clearance (geometric mean clearance [CL/F] range of 10.3 to 13.9 L/hr). The fraction of AG-348 excreted in urine ranged from 0.0145 to 0.0209 across the dose levels suggesting that renal excretion plays a minor role in the systemic elimination of AG-348.

[1] At the time of this document, results from Study AG348-C-002 in healthy volunteers have been unblinded, but the data have only partially been analysed and the Clinical Study Report is in preparation but not yet completed.

The preliminary repeat-dose PK of AG-348 at doses ranging from 15 mg every 12 hours (q12h) to 700 mg q12h also showed an increase in AG-348 exposure in a dose-proportional manner after the first dose. AG-348 exposure observed after repeated dosing for 14 days at doses of 120 mg every 24 hours (q24h) to 700 mg q12h were lower than observed on Day 1 after the first dose. The magnitude of the effect was greater at higher doses; lower doses of 15 mg q12h and 60 mg q12h did not show this effect. This observation may be related to the nonclinical finding that AG-348 is a potential inducer of human CYP3A4, an enzyme which biotransforms AG-348. It is possible that multiple doses of AG-348 leads to an increased rate of its own metabolism.

Evaluation of the effect of food on the PK of a single 700 mg dose of AG-348 in 5 subjects who were administered the drug fasting and then, after an appropriate wash-out period, re-administered the drug following ingestion of a standard US Food and Drug Administration (FDA) high fat meal, showed that food likely has a minimal effect on the PK of AG-348.

#### 5.2.2.2. Pharmacodynamics

After a single dose of AG-348, a decrease in the concentration of 2,3-DPG was observed at 3 hours post-dose, decreased in a dose-dependent manner to a minimum at 24-hour post-dose, and then returned to values similar to baseline by 72 to 120 hours post-dose. The mean decrease at 24 hours was approximately 300 µg/mL at the 700 through 2500 mg dose levels. Similar decreases were observed after the first dose of multiple doses and prior to doses in the MAD study. After the final dose of multiple doses, the concentration of 2,3-DPG returned to values similar to baseline between 72 and 120 hours post- dose.

After a single dose of AG-348, a minimal increase in the concentration of ATP was observed at 24 to 120 hours post-dose. In the MAD study, no increase was observed at 12 hours after the first dose; the concentration of ATP was increased on Day 8 to concentrations greater than in the single dose study, continued to trend upward on Day 11, and remained at a similar level through Day 14. The concentration of ATP remained elevated through 120 hours after the last dose on Day 14. The magnitude of the increase in ATP was similar across the dose range from 60 mg BID to 700 mg BID.

#### 5.2.2.3. Safety

Overall, AG-348 has been well-tolerated among healthy volunteers at doses that produced strong pharmacodynamic (PD) effects on 2,3-DPG and ATP.

After a single AG-348 dose, treatment-emergent adverse events (TEAEs) reported by > 1 subject at any time on study (either under fasted or fed conditions) included headache (22%), nausea (14%), and contact dermatitis and vomiting (each 6%). After repeated dosing of AG-348 for 14 days, TEAEs that occurred in > 5% of all AG-348-treated subjects across all cohorts included headache (13.9%), nausea (13.9%), vomiting (8.3%), decreased appetite (8.3%), feeling hot (8.3%), restlessness (8.3%), and dizziness, fatigue, vessel puncture site bruise, hyperhidrosis, dermatitis allergic, and drug eruption (5.6% each).

A dose-relationship was apparent with regard to the incidence of gastrointestinal events, primarily nausea and vomiting, with the incidence of such events increasing with increasing dose. Nausea and/or vomiting were observed only at doses ≥ 1400 mg in the single dose study and only at doses ≥ 700 BID in the MAD study. Nausea and vomiting were not observed at any dose ≤ 360 mg in either the single or multiple dose studies.

All but 1 TEAE reported to date has been mild or moderate (Grade 1 or 2) in intensity. The only Grade 3 TEAE was elevated liver transaminases (alanine aminotransferase [ALT]; aspartate aminotransferase [AST]) in a single subject in the MAD study treated with 700 mg AG-348 BID. The event was considered to be possibly related to AG-348, was declared a DLT, and led to study drug discontinuation, following which the elevated liver transaminases resolved. Altogether, 3 of 6 subjects treated with AG-348 at 700 mg BID discontinued study drug in the MAD study: the subject described above with Grade 3 elevated transaminases, and 2 others who withdrew themselves for nausea and vomiting. A fourth subject treated with AG-348 at 60 mg BID was discontinued from the study for a drug eruption. No AG-348-treated subject discontinued in the SAD study due to an adverse event (AE).

No deaths or other serious AEs (SAEs) have been reported in any clinical study of AG-348. Furthermore, no DLTs were observed after a single administration of AG-348 at doses of 30 mg to 2500 mg or after repeat administration of AG-348 for 14 days at daily doses of 15 mg to 700 mg BID except for the event of Grade 3 elevated liver function tests described above.

Due to preclinical observations pertaining to the potential for inhibition of the aromatase enzyme (see Section 5.2.1.4), the AG348-C-002 multiple dose study included assessment of baseline and serial measures of free and total serum testosterone and serum estradiol and estrone. The unblinded serum hormone data are undergoing analysis, but at least some male subjects treated with all doses of AG-348 demonstrated modest increases in androgenic hormones and decreases in estrogens, compatible with a potential signal of aromatase inhibition, whereas the males who received placebo did not. These changes were reversible upon cessation of dosing. There were too few female subjects in the study who received AG-348 to permit any definitive conclusions. Additional analyses are ongoing.

## 5.3. Study Rationale

Study AG348-C-003 is the first study that will be conducted in patients with PK deficiency. This study is primarily intended to evaluate the safety and tolerability and potential indicators of clinical activity of AG-348 administered for up to 24 weeks. This study will also evaluate the PK profile of AG-348 and its metabolite AGI-8702, the PD responses in ATP and 2,3-DPG following administration of AG-348, and the clinical activity of AG-348 in PK deficiency patients. Two previous double-blind, placebo-controlled clinical trials of AG-348 conducted in healthy adult male and female volunteers (AG348-C-001, a SAD study; and AG348-C-002, a MAD study) have established an acceptable safety and tolerability profile for AG-348 for up to 14 days of both once-daily (QD) and BID dosing at exposures that result in significant PD changes in whole blood levels of the glycolytic metabolites 2,3-DPG and ATP. Due to the potential for AG-348-mediated aromatase inhibition, dual-energy x-ray absorptiometry (DXA) scan (hip and spine) will be performed at Screening (if patient has not had prior DXA scan within 3 months of Day 1) to obtain T and Z scores. These data will serve as baseline measures of bone mineral density for all enrolling patients (see Section 7.1 for more details).

The target population of this study consists of adult males and females with a diagnosis of PK deficiency, who are anemic but non-transfusion dependent. Non-transfusion dependent patients are preferred for this study in order to reduce any potential confounding effect of transfusion therapies on evaluation of potential indicators of clinical activity and PD responses. The safety,

tolerability, and PK/PD findings in this study will form the basis for subsequent clinical development of AG-348.

The Extension Period is offered in order to allow for longer study of AG-348 in patients who experience a benefit and tolerate the drug.

### 5.3.1. Summary of Overall Safety Management Plan

Measures to minimize the risks to patients enrolled in the Core and Extension Periods have been taken with respect to the following study design elements:

- The initial doses for Arms 1 and 2 have been selected on the basis of safety and tolerability observations already carefully made in two predecessor clinical trials in adult healthy male and female volunteers;
- The specified inclusion/exclusion criteria have been carefully considered to avoid enrollment of subjects for whom exposure to the study drug might pose a hazard;
- A designated Data Review Team (DRT) consisting of the overall study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer has been established to meet regularly at approximately 6-week intervals throughout the duration of the Core Period, and approximately every 3 months during the Extension Period once all patients have completed the Core Period to review the accumulating study data and will exercise options to suspend enrollment to one or both of the initial two study dose arms, discontinue enrollment to one or both of the initial two study dose arms, adjust the dose of patients in one or both of the initial two study arms, and/or implement one new study dose arm. If one new dosing arm is implemented in the Core Period, the dose selected will not exceed 360 mg BID, the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy volunteers. Group cohort stopping rules for terminating enrollment into an arm based on the severity (Common Terminology Criteria for Adverse Events [CTCAE]v4.03 grade) and frequency of AEs are defined;
- Dose modification and stopping rules are defined for individual patients;
- Guidance for permitted, prohibited, and cautionary concomitant medications is specified based on the estimated potential for DDIs from hepatic cytochrome enzyme interactions with AG-348.
- Due to the potential for AG-348-mediated aromatase inhibition, bone mineral density will be monitored using DXA scans (hip and spine) at Baseline (if patient has not had prior DXA scan within 3 months of Day 1) and between Week 24 and Week 28.

In the event that any clear and unequivocal, previously unidentified/unexpected toxicities occur in pre-clinical toxicology studies, the Sponsor will notify the Investigators, Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs), and regulatory authorities. The Sponsor will also report these to the DRT for review and discussion of appropriate actions, which may include, but are not necessarily limited to:

- Amending the protocol to adjust the inclusion/exclusion criteria (e.g., to exclude patients with certain at-risk concurrent conditions); and/or
- Amending the protocol to adjust safety monitoring procedures (e.g., to require additional monitoring of specified AEs, physical examinations, clinical laboratory testing, ECG monitoring, or other testing as appropriate); and/or
- Adjusting the dose of an arm of the study as appropriate; and/or
- Adjusting the dose modification and/or stopping rules (Section 9.7); and/or
- Adjusting the patient withdrawal criteria (Section 8.6); or
- Terminating the trial.

# 6. TRIAL OBJECTIVES AND ENDPOINTS

## 6.1. Core Period

### 6.1.1. Primary Objective

The primary objective of the study is to:

- Evaluate the safety and tolerability of up to 24 weeks of AG-348 administration in patients with PK deficiency.

### 6.1.2. Secondary Objectives

The secondary objectives of the study are to:

- Evaluate the PK of AG-348 and the metabolite AGI-8702.
- Evaluate the PD response of ATP and 2,3-DPG after administration of AG-348.
- Evaluate indicators of clinical activity of AG-348 in patients with PK deficiency, including changes in hemoglobin (Hb), HCT, reticulocyte count, haptoglobin (Hp), carboxyhemoglobin (COHb), lactate dehydrogenase (LDH), total and indirect bilirubin, erythropoietin (EPO), ferritin, and transferrin saturation (serum iron/iron binding capacity).

## 6.2. Extension Period

### 6.2.1. Primary Objective

The primary objective of the study is to:

- Evaluate the safety and tolerability of up to 30 months of AG-348 administration in patients with PK deficiency.

### 6.2.2. Secondary Objectives

The secondary objectives of the study are to:

- Evaluate the PK of AG-348 and the metabolite AGI-8702.
- Evaluate the PD response of ATP and 2,3-DPG after administration of AG-348.

- Evaluate indicators of clinical activity of AG-348 in patients with PK deficiency, including changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation (serum iron/iron binding capacity).

## 6.3. Study Measures and Endpoints

### 6.3.1. Safety Measures and Endpoints

Safety will be evaluated by:

- Monitoring of AEs, including determination of SAEs and AEs leading to discontinuation; safety laboratory parameters (hematology, chemistry, urinalysis, coagulation); physical examination findings; vital signs (VS); 12 lead electrocardiograms (ECGs); and DXA scans. Adverse events will be graded using CTCAE, Version 4.03. Serum sex hormone levels (testosterone [total and free], estrone, and estradiol), bone turnover markers (serum osteocalcin-N-mid and serum C-terminal telopeptide [CTX]), 25-hydroxy vitamin D2 and D3, total cholesterol, high-density lipoprotein-cholesterol (HDL-C), and triglycerides will be monitored for evidence of potential inhibition of aromatase by AG-348. Menstruating female patients will also keep a paper-based menstrual cycle diary throughout the Core and Extension Periods.

### 6.3.2. Clinical Activity Measures and Endpoints

- Monitoring of potential indicators of clinical activity will include evaluating changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation.

### 6.3.3. Pharmacokinetic and Pharmacodynamic Measures and Endpoints

The PK and PD profile of AG-348 will be evaluated by:

- Approximately the first 10 patients treated during the Core Period, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 15.1, Table 7. The remainder of treated patients will undergo limited PK sampling as detailed in Appendix 15.1, Table 8. During the Core Period, serial blood sampling for determination of concentration-time profiles of AG-348 and its metabolite AGI-8702 will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of AG-348 and AGI-8702 will be obtained. During the Extension Period, predose PK samples will be drawn for the measurement

of trough levels of AG-348 and AGI-8702 at each study visit (every 3 months; Appendix 15.1, Table 6). AG-348 and AGI-8702 will be analyzed using qualified assays to determine concentrations in plasma. Pharmacokinetic parameters on Day 1 and Day 15 will be computed using standard non-compartmental methods based on observed plasma AG-348 and AGI-8702 concentrations.

- Pharmacodynamic assessments during the Core Period will include 2,3-DPG, ATP (secondary objectives), [redacted] Approximately the first 10 patients treated during the Core Period will undergo extensive PD sampling as detailed in Appendix 15.1, Table 7. The remainder of treated patients will undergo limited PD sampling as detailed in Appendix 15.1, Table 8. During the Core Period, serial blood sampling for determination of levels of ATP and, 2,3-DPG will be conducted following the first dose and the morning Day15 dose, and additional trough levels of ATP and 2,3-DPG will be obtained. During the Extension Period, predose PD samples will be drawn for the measurement of trough levels of 2,3-DPG, ATP, [redacted] at each study visit (every 3 months; Appendix 15.1, Table 6). Adenosine triphosphate and 2,3-DPG will be analyzed using qualified assays to determine concentrations in whole blood. PD parameters on Day 1 and Day 15 will be computed based on observed whole blood ATP and 2,3-DPG concentrations.

[redacted]

# 7. INVESTIGATIONAL PLAN

## 7.1. Overall Study Design

Study AG348-C-003 is a Phase 2, open-label, two-arm, multicenter, randomized, dose-ranging study in adult patients with PK deficiency; the study will be divided in to a Core Period and an Extension Period. During the Core Period, patients will receive multiple doses of AG-348 for up to 24 weeks; patients who are eligible can enter the Extension Period to receive AG-348 for up to 2 years following the end of the Core Period. Patients with PK deficiency confirmed by RBC PK enzymatic assay performed at Screening will be eligible to participate in this study. At Week 25, patients who safely tolerate AG-348 and demonstrate clinical activity of AG-348 may be eligible to immediately roll over to the Extension Period for continued treatment. Patients who complete treatment at the end of the Core Period (24 weeks) will undergo follow-up assessment 4 weeks after the last dose of study drug. If a patient discontinues at any other time (including early discontinuation or discontinuation during the Core or Extension Period), follow-up assessments will be conducted 4 weeks after discontinuation. Patients with toxicity suspected to be related to study drug will continue follow-up until the AE resolves, is declared chronic by the Investigator, or the patient is lost to follow-up.

For the Core Period, up to 25 patients will be initially randomized on an open-label, 1:1 basis to each of two BID doses of AG-348 (up to 50 patients; see Figure 3, Study Schema). The dose of Arm 1 is 300 mg of AG-348 administered orally q12h (BID). The dose of Arm 2 is 50 mg of AG-348 administered orally q12h (BID). Randomization will be stratified by *PKR* mutation in order to maintain balance as much as possible across the dose arms for the specific mutations expected to be most frequently enrolled. The PKR mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H) and all other mutations ("other"). Mutation status is defined by the presence of at least one of the indicated mutations; patients with more than one stratified mutation will be assigned based on Sponsor's discretion.

The doses for each arm of the Core Period have been selected from the AG348-C-001 SAD study and AG348-C-002 MAD studies in healthy adult volunteers to represent the range of doses/exposures that were safely tolerated and resulted in maximal or near maximal PD effects on 2,3-DPG and ATP.

Because PK deficiency is a rare disease with a limited eligible patient population and because the underlying pathophysiology and clinical phenotype of affected patients is heterogeneous due to the wide variety of mutations in PKR that cause the disease, it is deemed important to focus closely on dose findings in this first-in-patient study. Therefore, in addition to initiating this study with 2 different doses of AG-348 administered q12h, a DRT will be established to review study data on a frequent basis and adapt the study design, dose and schedule of AG-348 as indicated.

The DRT will monitor safety on an on-going basis and meet at regular intervals of approximately every 6 weeks, or *ad hoc* as necessary, for as long as any patients are still in the Core Period to review AEs, VS, clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and ECGs. The DRT will also review available PK/PD data and indicators of clinical activity (e.g., changes from baseline in Hb). These DRT meetings will also include data review

for all patients that may be under treatment in the Extension Period. If there are no patients still being treated in the Core Period, and the only patients on treatment are those in the Extension Period, then the frequency of the DRT meetings will reduce to approximately every 3 months in order to match the frequency of patient visits (and new data collection) in the Extension Period. The DRT will be comprised of the study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer.

Beginning 6 weeks after the first patient is dosed in the Core Period or *ad hoc* as necessary, and proceeding according the schedule indicated above (approximately every 6 weeks during the Core Period, approximately every 3 months during the Extension Period once all patients have completed the Core Period), the DRT will review cumulative safety data, available PK/PD data, and clinical activity data. Based on the DRT's recurring, the DRT may exercise one or more of the following options during the Core Period:

- Continue treatment and enrollment in existing arms without change.
- Add 1 new dose arm (Arm 3) to enroll up to 25 patients at a dose to be determined; the dose for Arm 3 may be lower or higher than Arm 1 and Arm 2 doses, but will not exceed 360 mg q12h; and the dose regimen may be less frequent than q12h.
- Terminate or suspend enrollment to allow further review of clinical data in Arm 1 and/or Arm 2 (and/or potential Arm 3). Enrollment in an arm could be terminated or suspended to allow further review, for example, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity.
- Re-assign patient's doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the patients in the terminated arm will remain in their original arm, i.e., they will not count towards the enrollment quota of the arm whose dose and schedule is being adopted.
- Implement specific genotype restrictions to enrollment in one or more arms to ensure representation of patients with genotypes of greatest clinical relevance.

The DRT may exercise one or more of the following options during the Extension Period:

- Continue treatment without change;
- Re-assign patients' doses and schedule to an existing dose and schedule that has been determined to be safer, and/or have a better PD response, or produce signs of clinical activity;
- Terminate or suspend treatment to allow further review of clinical data (eg, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity).

The data that the DRT will review to make these decisions is expected to include, but are not necessarily limited to, the following:

- *Safety Observations:* all AEs; VS, clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and ECGs;
- *PK and PD Observations:* including changes in 2,3-DPG and ATP;

- *Indicators of Clinical Activity:* including changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, EPO, total and indirect bilirubin, ferritin, and transferrin saturation.

If a third dose arm is implemented, the dose of AG-348 selected will not exceed 360 mg BID, as this was the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy volunteers. The PK/PD sampling schedule in a potential third dosing arm will be determined by Sponsor's discretion and may follow either the extensive or limited PK/PD sampling schedules as specified.

The DRT will monitor the safety data in an ongoing manner as described and may make a decision to terminate enrollment in an arm if unacceptable AEs are observed, PD activity is limited, and/or markers of clinical activity indicate no effect. Unacceptable AEs are defined as ≥ Grade 3 AEs (using National Cancer Institute (NCI) CTCAEv4.03) that are assessed as at least possibly related to AG-348, with the exception of Grade 3 headache, hot flash/flushing, nausea, vomiting, and/or diarrhea that is transient in duration (< 24 hours) or able to be medically managed to ≤ Grade 2 within 24 hours. The DRT may also exercise discretion to terminate enrollment in an arm if it observes a frequency of Grade 2 AEs that would make it difficult to support long-term dosing.

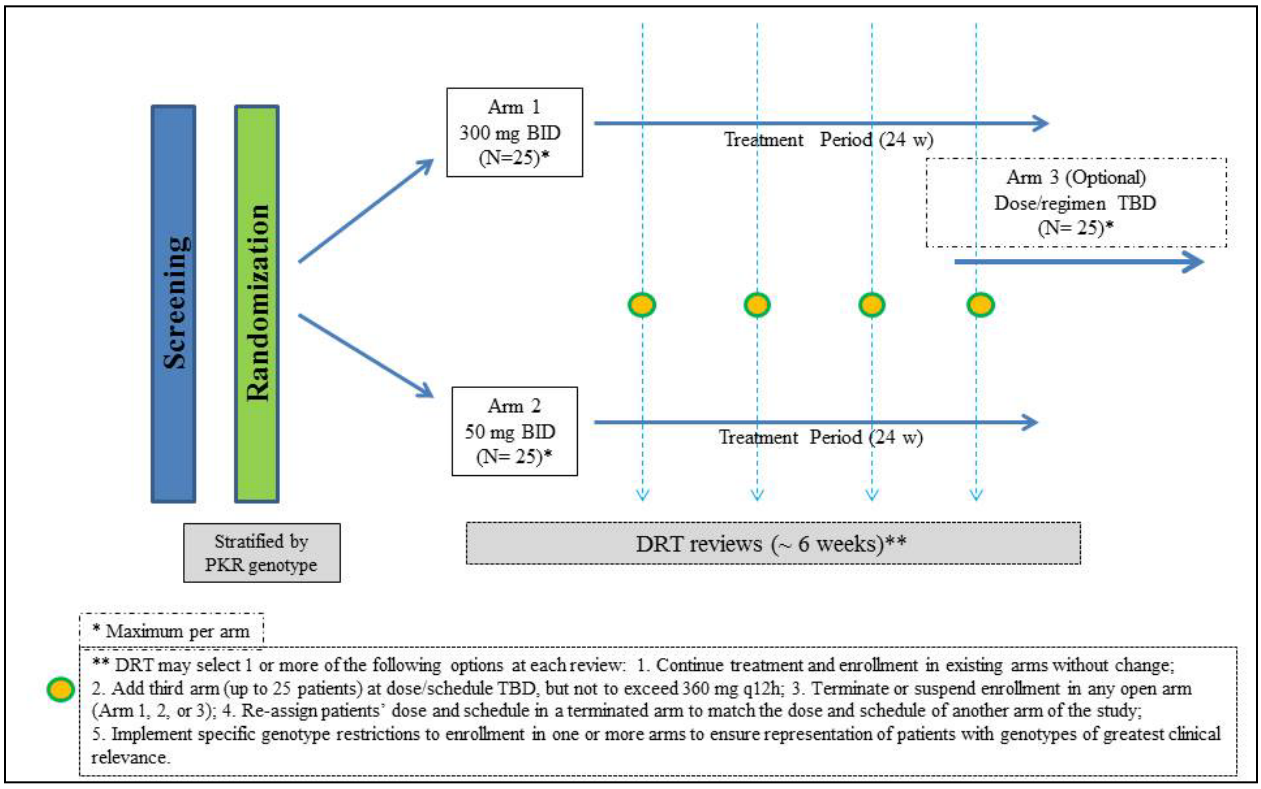
Patients in the Extension Period will continue on the dose they were randomized to in the Core Period, unless the DRT had reason to establish a different dose/schedule during the course of the Core Period (the DRT will not propose a dose higher than 360 mg q12h).

Due to the potential for AG-348-mediated aromatase inhibition, DXA scan (hip and spine) will be performed at Screening (if patient has not had prior DXA scan within 3 months of Day 1) to obtain T and Z scores. These data are intended to serve as a baseline measure of bone mineral density for all enrolling patients, and are deemed of particular importance for those who may enter the longer term Extension Period after completing 24 weeks of treatment (Core Period). All patients will have a second DXA scan in the interval between Weeks 24 and 28 for the Core Period. Patients in the Extension Period will have additional DXA scans at Months 18 and 30..

As the number of enrolling arms changes in the study (for example, from 2 to 3), the randomization scheme will adjust to enable balanced randomization into each actively accruing arm. Randomization and stratification will cease in the event that only a single arm is left enrolling.

Depending on possible early termination of 1 or both of the initial 2 arms, or the addition of a third arm, the study could enroll up to a maximum of 75 patients.

**Figure 3: Study Schema: Core Period**




Abbreviations: BID (q12h) = twice-daily (every 12 hours); DRT = data review team; PKR = pyruvate kinase red blood cell isoform; TBD = to be determined; w = weeks.

The Investigator will monitor all patients for safety and tolerability. Modification of an individual patient's dose of AG-348 will be based on AEs and observed changes in Hb levels as detailed in Section 9.7.1 and Section 9.7.2.

Screening assessments will occur within 28 days prior to the first dose of study treatment. During the Core Treatment period, patients will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12), and monthly through Week 24 (Weeks 16, 20, and 24). Patients who safely tolerate AG 348 through Week 24 (Core Period) may be eligible to immediately enter the Extension Period for continued treatment. Study visits for safety and clinical activity assessments will occur approximately every 3 months during the Extension Period for up to 2 years after the end of the Core Period. All patients will undergo a follow-up assessment 4 weeks after the last dose of AG-348, regardless of whether this was due to early discontinuation, the last dose in the Core Period for a patient who chooses not to continue in the Extension Period, or the last dose of the Extension Period.

Safety assessments will include monitoring of AEs, including determination of SAEs and AEs leading to discontinuation; safety laboratory parameters (e.g., hematology, serum chemistry, coagulation studies, and urinalysis); physical examination findings; VS; 12 lead ECGs, and DXA scans. Additional safety assessments will include monitoring of sex hormone levels (testosterone [total and free], estrone, and estradiol), bone turnover markers (osteocalcin-N-mid and CTX), 25-hydroxy vitamin D2 and D3 levels, total cholesterol, HDL-C, and triglycerides. Follow-up

assessments will be conducted on Day 197 (Week 28) for patients who do not enter the Extension Period and will include physical examination, weight, performance status, VS, 12-lead ECGs, laboratory evaluations (hematology, Hp, EPO levels, serum chemistry, coagulation studies, urinalysis; lipids, hormonal testing), AEs, and transfusion record. These follow-up assessments will be performed approximately 4 weeks after discontinuation of AG-348 for patients who discontinue prior to completion of the Core Period and for those who discontinue in the Extension Period. Menstruating female patients will also be required to keep a paper-based menstrual cycle diary throughout the study.

Pharmacokinetic assessments will include serial blood sampling for PK profiles of AG-348 and its metabolite AGI-8702. Pharmacodynamic evaluations will include serial blood sampling for determination of levels of ATP and 2,3 DPG. Extensive PK/PD sampling will be conducted on the first approximately 10 patients total treated in Arms 1 and 2 of the Core Period (see Appendix 15.1, Table 7) while limited PK/PD sampling will be conducted on the remainder of treated patients (see Appendix 15.1, Table 8). Limited trough sampling will be conducted every 3 months during the Extension Period (Appendix 15.1, Table 6).

## 7.2. Justification of the Study Design

The primary and secondary objectives of this study are to evaluate the safety, tolerability, PK and PD, and indicators of clinical activity of AG-348 in patients with PK deficiency. The choice of dose and schedule of administration of AG -348 for Arms 1 and 2 was based on the highest safely tolerated dose (Arm 1: 300 mg BID) and the lowest dose with potentially relevant PD activity (Arm 2: 50 mg BID) from the forerunner AG348-C-002 MAD study in healthy volunteers. Decisions regarding continuing enrollment and treatment in these initial dose arms and/or implementation of an additional dose arm will be based on DRT review of safety, PK and PD data, and indicators of clinical activity collected from all patients treated in Arm 1 and Arm 2. This design was chosen to minimize risk to patients while allowing evaluation of safe and pharmacologically active dose levels of AG-348, and to allow the necessary flexibility to adjust dose and schedule should the safety, tolerability, PK, and/or PD be different in patients with PK deficiency compared with healthy volunteers.

Additional safety measures intended to minimize risk to patients include monitoring of AEs by the DRT and specified provisions for individual patient dose modification as needed for safety and (potentially) large increases in Hb level (Section 9.7.1 and Section 9.7.2). Measures intended to maximize the opportunity for patients with demonstrated safety and tolerability to continue to derive benefit from any observed clinical activity of AG-348 include the option for continued treatment in the Extension Period.

A comprehensive series of safety evaluations, including laboratory parameters, physical examinations, VS, 12-lead ECGs, and monitoring for SAEs and AEs, will be conducted to evaluate the safety profile of AG-348 and to aid in the determination of the recommended dose for continued development.

The study includes serial blood sampling across at least two different doses of the study drug to assess its PK and PD profiles.

Consistent with the design of many Phase 2 studies, preliminary evaluation of the potential PD and clinical activity of AG-348 are secondary objectives of this study. The latter will include assessments of the PK/PD relationship between AG-348 and the biomarkers ATP and 2,3-DPG, [redacted]

## 7.3. Rationale for the Starting Dose, Dose Range, and Duration of Dosing

Prior to execution of this study, Agios conducted two clinical studies of AG-348 in healthy volunteers, including a SAD study (AG48-C-001) and a MAD (14 day q12h) study (AG348-C-002). Available details of these studies are discussed in the current Investigator's Brochure (IB). Between these two studies, 72 healthy human subjects have been dosed with AG-348. *In vitro* investigations, also reported in the IB, had previously demonstrated that AG-348 increased the activity of wild-type PKR approximately to the same extent as it did a series a recombinant mPKRs. Therefore it was deemed reasonable to study the safety, tolerability, PK, and PD of AG-348 in healthy subjects in a controlled Phase 1 setting as a more efficient means of obtaining information than in the actual rare disease population of patients with PK deficiency.

The MAD study demonstrated that the exposures produced by AG-348 doses from 60 mg q12h to 360 mg q12h (including 120 mg q24h) resulted in maximal changes from baseline for the PD markers 2,3-DPG (reduction from baseline) and ATP (increase from baseline). The exposures resulting from doses less than 60 mg q12h were of lesser magnitude and the exposures resulting from doses greater than 360 mg q12h were of no greater magnitude than the aforementioned range. Therefore the starting doses for this first dose ranging study in patients with PK deficiency were selected to be 300 mg q12h (Arm 1) and 50 mg q12h (Arm 2). These doses were demonstrated to be safe and tolerable in the healthy volunteer studies. The availability of ATP is proposed as being critical for optimally maintaining RBC membrane integrity (see Section 5.1). The dose ranges from 50 mg q12h to 300 mg q12h may result in clinically effective modulation of PKR in PK deficiency patients if the mutated enzyme is responsive to AG-348 in a similar manner to the wild-type enzyme in healthy subjects. However, there are many different mutations in PKR that result in PK deficiency, and these mutations produce variable effects on the enzyme in terms of catalytic activity and thermal stability. It is not known if different mutations will respond clinically in a similar manner to the same exposure to AG-348. Therefore, it is prudent to study the range of safe and pharmacodynamically relevant doses as specified in this study, and to allow flexibility for the DRT to analyze the evolving study data to adapt the dose and schedule of administration of AG-348 to produce the optimal combination of safety, tolerability, and PD, and, potentially, clinical response.

**Justification for Duration of Dosing**

The initial treatment duration of 24 weeks (6 months) for the Core Period was chosen for this proof-of-concept trial for 2 principal reasons: 1) to begin establishing a safety database addressing the chronic administration of AG-348; and 2) to allow sufficient time for clinical response to treatment to appear.

It is anticipated that this treatment, if successful, may be taken for life, as PK deficiency is a genetically determined inborn error of metabolism. Therefore, it is important to begin to investigate the long-term safety of the treatment. As will be discussed below, the safety package supports the treatment duration of 6 months.

Red blood cell turnover is typically 120 days (4 months), although it may be shorter in some populations of pyruvate kinase deficient RBCs (Mentzer, et al. 1971). Different PKR mutations produce a variety of physiologic consequences. In mutations where normal or nearly normal levels of PKR protein persist, but the protein's catalytic function is impaired, an improvement in PKR functional activity might be seen relatively quickly upon exposure to AG-348. However, in cases where the PKR mutation results in an unstable mutant, PKR protein levels may be low and additional time may be required for stabilization of the newly synthesized mutant enzyme in developing bone marrow erythroblasts so that eventually more mature RBCs may be produced with more nearly normal levels of functional PKR protein.

This study plans up to 24 weeks of dosing with AG-348 during the Core Period with the possibility of continued dosing beyond 24 weeks during the Extension Period in patients for whom AG-348 is safely tolerated and demonstrates clinical activity. The International Conference on Harmonisation (ICH) Guideline M3(R2) on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals, recommends that clinical trials of a duration between 2 weeks and 6 months should be supported by repeat-dose toxicity studies of at least equivalent duration, and clinical trials lasting beyond 6 months should be supported by 6-month rodent and 9-month non-rodent studies. This guidance allows for clinical trials of longer duration to be initiated based on 3 months of nonclinical data, and for clinical dosing in serious or life-threatening indications to be extended based on complete chronic rodent data and in-life and necropsy data in the non-rodent (and complete histopathology in the non-rodent provided within an additional 3 months).

For the current investigational product (AG-348), 13-week, repeat dose toxicology studies in the rat and monkey have been completed and are summarized in Section 5.2.1.4 of this protocol and in the current Investigator Brochure prepared to support initiation of this clinical study. Considering that PK deficiency is a serious condition with an unmet medical need, it is appropriate to initiate this clinical study with the available nonclinical data. Six-month rodent and nine-month monkey toxicology studies were initiated in January 2015, and the Sponsor will report the results of these studies in each applicable regulatory region as required before any patients will be treated for greater than 6 months (i.e., enter the Extension Period).

## 7.4. Criteria for Study Termination

This study may be prematurely terminated if, in the opinion of the Sponsor, there is sufficient reasonable cause. In the event of such action, written notification documenting the reason for study termination will be provided to each Investigator.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to patients;
- Insufficient adherence to protocol requirements;
- Plans to modify, suspend, or discontinue the development of the study drug;

- Other administrative reasons.

Should the study be closed prematurely, all study materials must be returned to the Sponsor or the Sponsor's designee. Patients who have participated in the Extension Period may be able to screen for eligibility in a study for a second generation allosteric PKR activator, if such a compound is available at that time, and after a suitable washout period has been observed.

# 8. STUDY POPULATION

## 8.1. Number of Patients

Up to approximately 75 patients may be enrolled in this study.

## 8.2. Inclusion Criteria

For entry into the Core Period, patients must meet all of the following criteria during the Screening or other specified period:

1. Signed written informed consent obtained prior to performing any study procedure, including screening procedures.
2. Male or female, aged 18 years and older.
3. Known medical history of PK deficiency.
4. All patients must have documented clinical laboratory confirmation of PK deficiency by RBC pyruvate kinase enzymatic assay performed at Screening by a designated central laboratory. Patients with prior documentation of PK deficiency by RBC enzymatic assay will have a reconfirmation of this result during Screening as a condition of enrollment.
    a. In the event that a patient's screening pyruvate kinase enzymatic assay is negative (i.e., shows normal pyruvate kinase activity), the patient will be eligible for enrollment if the genotyping shows a mutant genotype that has been previously documented in the literature to be associated with PK deficiency. If the genotyping shows a previously undescribed mutation in the PKR gene, then the eligibility for enrollment will be determined on a case-by-case basis by the Coordinating Investigator and Medical Monitor in discussion with the Investigator. If no mutation is defined, then the patient will not be eligible.
5. All patients must have genotypic characterization of the mutant PKR gene performed by the designated central laboratory at Screening.
6. All patients must have genotypic characterization of the UGT1A1 gene performed by a designated central laboratory to document whether they may have underlying Gilbert's Disease. Patients with Gilbert's Disease are eligible to enroll.
7. Males must have Hb $\leq$ 12.0 g/dL; females must have Hb $\leq$ 11.0 g/dL.
8. All patients must be considered transfusion independent as defined by: no greater than 3 units of RBCs transfused in the 12-month period up to the first day of study dosing and no transfusions within 4 months of first day of study dosing. Patients who have received more transfusion support than described above will be evaluated for eligibility on a case-by-case basis by the Medical Monitor.
9. Eligible patients may still have their spleens in place, or may have undergone prior splenectomy. For splenectomized patients:
    a. Must have undergone their procedure at least 6 months prior to Screening.

b. Must be current in their vaccinations for Pneumococcal Conjugate (PCV13), Pneumococcal Polysaccharide (PPSV23), Quadrivalent Meningococcal vaccine, and *Haemophilus influenzae* type b as recommended by Centers for Disease Control and Prevention's (CDC) Advisory Committee on Immunization Practices (ACIP) or immunization advisory groups in Canada and the European Union (for patients enrolled in Canada and the EU). [http://www.cdc.gov/vaccines/schedules/downloads/adult/adult-schedule.pdf] [Any missing vaccinations may be administered during the screening period. If the patient requires both PCV13 and PPSV23, PCV13 must be given before PPSV23, if possible. Administration of PPSV23 should follow PCV13 by at least 8 weeks; it is permissible to give PCV13 during Screening followed by PPSV23 following the initiation of AG-348 treatment.]

10. Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 2 (Appendix 15.2).
11. Patients must be taking at least 1 mg of folic acid daily for at least 21 days prior to first dose and continued daily during study participation.
12. Adequate organ function, defined as:
    a. Serum AST and ALT ≤ 1.5 × upper limit of normal (ULN) (unless the increased AST is assessed by the Investigator as due to hemolysis).
    b. Normal or elevated levels of serum bilirubin. In patients with serum bilirubin > ULN, the elevation must be attributed to hemolysis with or without Gilbert's syndrome and must not be choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease.
    c. Serum creatinine ≤ 1.25 × ULN. If serum creatinine > 1.25 × ULN, then 24-hour measured or calculated (Cockcroft-Gault) glomerular filtration rate (GFR) ≥ 60 mL/min.
    d. Absolute neutrophil count (ANC) > 1.0 × 109/L.
    e. Platelet count ≥ 100 × 109/L.
    f. Activated partial thromboplastin time (aPTT) and international normalized ratio (INR) ≤ 1.25 × ULN, unless the patient is receiving therapeutic anticoagulants.
13. Women of childbearing potential (WOCBP) must agree to abstain from sexual intercourse or to use an acceptable/effective method of contraception (i.e., condom plus spermicide, condom plus oral contraceptive, condom plus intrauterine device [IUD], condom plus diaphragm with spermicide) from as soon as feasible during the Screening period until 30 days following the last dose of AG-348. Abstinence is an acceptable method only when this is in line with the normal life style of the patient, meaning that the patient plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence, e.g. calendar, sympathothermal and post-ovulation methods, and withdrawal are not acceptable methods of contraception.
    a. WOCBP include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation,

and/or bilateral oophorectomy) or is not post-menopausal. Post-menopausal is defined as:

i. Amenorrhea ≥ 12 consecutive months without another cause, and a documented serum follicle stimulating hormone (FSH) level > 35 mIU/mL;

ii. Amenorrhea≥ 12 consecutive months in women ≥ 62 years old (FSH testing is not required).

14. WOCBP must have a negative serum or urine pregnancy test within 72 hours before start of AG-348 dosing.
15. Women must not be breastfeeding.
16. Male patients, with the exception of those who have undergone vasectomy at least 6 months prior to Screening, must agree to abstain from sexual intercourse or, if sexually active, to use a condom with spermicide as contraception (regardless of their female partner's childbearing potential or their partner's use of their own contraception) from Day 1 of dosing until 30 days following the last dose of AG-348. Abstinence is an acceptable method only when this is in line with the normal life style of the patient, meaning that the patient plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence, e.g. selective timing of intercourse based on partner's calendar, sympathothermal and post-ovulation methods, and withdrawal are not acceptable methods of contraception.

For entry into the Extension Period, patients must meet the following criteria:

17. Signed written informed consent obtained prior to performing any study procedure during the Extension Period.
18. Patient must have completed 24 weeks of treatment during the Core Study and tolerated AG-348 (defined as having completed 24 weeks with or without permitted dose modifications).
19. The patient’s treating Investigator agrees that there is a potential for clinical benefit to continued treatment and recommends participation in the Extension Period
20. The Sponsor's designated Medical Monitor or Responsible Medical Officer approves the patient’s participation in the Extension Period
21. As applicable, the patient must agree to continue to follow the same sexual abstinence/contraception rules as stated in Inclusion Criteria 13 and 16.

## 8.3. Exclusion Criteria

Patients who meet any of the following criteria at Screening or prior to dosing on Day 1 will not be enrolled in Core Period:

1. Hemoglobin level > 12.0 g/dL if male; Hb > 11.0 g/dL if female.
2. Additional diagnosis of any other congenital or acquired blood disorder, including glucose-6-phosphate-dehydrogenase (G6PD) deficiency, or any other hemolytic anemia process except for mild allo-immunization as a consequence of transfusion therapy.

3. Iron overload (hemosiderosis or concurrent hemochromatosis) sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac, hepatic, or pancreatic insufficiency.
4. Prior bone marrow or stem cell transplant.
5. Clinically symptomatic cholelithiasis or cholecystitis. (Prior cholecystectomy is not exclusionary. Patients with symptomatic cholelithiasis or cholecystitis may be rescreened once the disorder has been treated and clinical symptoms have resolved.)
6. Currently enrolled in another therapeutic clinical trial involving on-going therapy with any investigational or marketed product or placebo. Concurrent participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480) is permitted.
7. Exposure to any investigational drug, device, or procedure within 28 days prior to Screening.
8. Concurrent medical condition that could compromise participation in the study such as:
    a. Poorly controlled hypertension (defined as systolic blood pressure (BP) > 150 mm Hg or diastolic BP > 90 mm Hg) refractory to medical management.
    b. History of recent (within < 6 months from Screening date) congestive heart failure; myocardial infarction or unstable angina pectoris; or hemorrhagic, embolic, or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.
    c. Currently active infection requiring the use of parenteral anti-microbial agents or that is ≥ Grade 3 (CTCAEv4.03) within 6 months of first dose.
    d. A pattern or frequency of post-splenectomy sepsis that in the assessment of the Investigator could reasonably be expected to interfere with the ability of the patient to complete the 24 week study participation.
    e. Positive test for hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV) antibody with signs of active Hepatitis B or C virus infection.
    f. Positive test for human immunodeficiency virus (HIV) 1 or 2 antibody.
    g. Diabetes mellitus judged to be in poor control by the Investigator or requiring > 3 anti-diabetic agents counting insulin; use of insulin *per se* is not exclusionary.
    h. History of any primary malignancy with the exception of: curatively treated non-melanomatous skin cancer; curatively treated cervical or breast carcinoma *in situ*; or other primary tumor treated with curative intent and no known active disease present and no treatment administered during the last 3 years.
9. Undergone major surgery within 6 months of first dose.
10. Current or recent history of psychiatric disorder that in the opinion of the Investigator or Medical Monitor could compromise the ability of the patient to cooperate with study visits and procedures.
11. Use of any of the restricted list of products known to strongly inhibit CYP3A4 metabolism (Appendix 15.4, Table 9) within 5 days prior to Day 1 dosing; or to strongly

induce CYP3A4 metabolism (Appendix 15.4, Table 10) within 28 days prior to Day 1 dosing; or to strongly inhibit P-gp transporter (Appendix 15.4, Table 11) within 5 days prior to Day 1 dosing; or digoxin within 5 days prior to Day 1 dosing. For patients who require chronic inhaled glucocorticoid therapy, Investigators should confer with the Medical Monitor for additional guidance.

12. Serum bilirubin > ULN attributable to factors other than hemolysis and/or Gilbert's syndrome.
13. Male patients with heart-rate corrected QT interval -Fridericia's method (QTcF) interval > 450 msec, or female patients with QTcF interval > 470 msec with the exception of patients with a left bundle branch block (LBBB). Medical Monitor approval needed in patients with a LBBB.
14. Cardiac dysrhythmias judged as clinically significant by the Investigator or requiring therapy with drugs that are primarily substrates of CYP3A4.
15. History of allergy to sulfonamides if characterized by acute hemolytic anemia, anaphylaxis, or rash of erythema multiforme type or Stevens-Johnson syndrome.
16. Any other medical or psychological condition deemed by the Investigator to be likely to interfere with a patient's ability to understand and sign informed consent; cooperate with study visits, tests, and procedures; or otherwise safely and reliably participate in the study.

Patients will not be allowed to enter the Extension Period if:

17. The patient experienced AEs during the Core Period that are considered by the treating Investigator or the Sponsor's designated Medical Monitor or Responsible Medical Officer to pose a significant safety risk to the patient if treatment were to be extended.

## 8.4. Patient Identification and Registration

Patients who are candidates for enrollment into the study will be evaluated for eligibility by the Investigator to ensure that the inclusion and exclusion criteria (see Section 8.2 and Section 8.3, respectively) have been satisfied and that the patient is eligible for participation in this clinical study. The site will submit to the Sponsor an Eligibility form for each eligible patient and the Medical Monitor will confirm eligibility for all patients prior to receipt of the first dose of AG-348.

## 8.5. Patient Randomization

Patients who have been confirmed as eligible will be randomized in an equal ratio to a treatment arm (e.g., 1:1 or 1:1:1 depending on which arms are open). The site will provide a request for randomization form (including the patient's confirmed genotype) to the study Medical Monitor. The randomization will be stratified by PKR mutation in order to maintain balance across the dose arms for the specific mutations expected to be most frequently enrolled. The PKR mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H) and all other mutations ("other"). Since this is an open label study, randomization will not be blinded.

Please refer to the study manual for the randomization procedure.

## 8.6. Patient Withdrawal Criteria

Patients have the right to withdraw from the study at any time for any reason. Patients will be withdrawn from study-related procedures and treatments under the following conditions:

- Withdrawal of consent;
- Experiences unacceptable toxicity;
- Development of an intercurrent medical condition that precludes further participation in the trial;
- Patient requires use of a prohibited concomitant medication (Section 9.11.2);
- Investigator decision;
- Protocol violation: non-adherence to protocol requirements;
- Pregnancy;
- Lost to follow-up.

Should a patient decide to withdraw, all efforts will be made to complete and report the protocol-defined study observations up to the time of the patient's withdrawal as completely as possible and to determine the reason for withdrawal.

In the event a patient is withdrawn from the study, the Medical Monitor must be informed. If there is a medical reason for withdrawal, the patient will remain under the supervision of the Investigator until satisfactory health is returned.

When a patient withdraws from the study, the primary reason for discontinuation must be recorded in the appropriate section of the electronic case report form (eCRF) and all efforts will be made to complete and report final study observations as thoroughly as possible.

All AEs should be followed until resolution or for a period of 30 days from the last dose of study drug, whichever is shorter. Patients with toxicity suspected to be related to study drug will continue follow-up until the AE resolves, is declared chronic by the Investigator, or the patient is lost to follow-up.

## 8.7. Replacement of Patients

Patients who drop out of the Core Period prior to completing the first 12 weeks of assigned dosing for reasons other than AEs may be replaced at the Sponsor's discretion.

# 9. STUDY TREATMENT

## 9.1. Description of Study Drug

AG-348 sulfate hydrate capsules will be provided as 5 mg, 25 mg, or 100 mg (free-base equivalent) of AG-348 sulfate hydrate without excipients in dark green opaque (5 mg), Swedish orange (25 mg), or white opaque (100 mg) gelatin capsules (size 2 capsules for all dose strengths).

All study drugs are for investigational use only and are to be used only within the context of this study. All study drug products will be supplied by the Sponsor. Please see the Investigator's Brochure for further details regarding study drug.

## 9.2. Study Drug Packaging and Labeling

AG-348 sulfate hydrate capsules are packaged in white, high-density polyethylene (HDPE) induction sealed bottles with a child-resistant screw cap.

Packaging and labeling will be prepared to meet all regulatory requirements.

## 9.3. Study Drug Storage

AG-348 sulfate hydrate drug capsules must be stored at room temperature of 15 to 30°C (59 - 86°F).

All study drug products must be stored in a secure, limited-access location and may be dispensed only by the Investigator or by a member of the staff specifically authorized by the Investigator.

## 9.4. Method of Assigning Patients to Treatment

Up to a maximum of 25 patients will be randomized to any one of the dosing arms in this study. Randomization will be balanced 1:1 or 1:1:1, depending on whether 2 or potentially 3 arms are open, and will be stratified by PKR mutation (see Section 8.5). The dose and schedule of AG-348 each patient receives will be dependent upon which dose arm is open for enrollment when the patient qualifies for and is randomized into the study. Patients in the Extension Period will continue on the dose they were randomized to in the Core Period, unless the DRT had reason to establish a different dose/schedule during the course of the Core Period (the DRT will not propose a dose higher than 360 mg q12h).

## 9.5. Blinding

This is an open-label study; no blinding methods will be used.

## 9.6. Study Drug Preparation and Administration

For the initial two treatment arms, (Arm 1 and Arm 2) in the Core Period, AG-348 will be administered orally BID (approximately q12h with a minimum of 10 hours between doses) over a 24 week treatment period. Starting with Day 1, dosing will be continuous; there will be no rest

periods. Patients who do not meet any of the treatment withdrawal criteria (see Section 8.5) may continue treatment for the entire 24-week treatment period.

Patients will be dispensed the appropriate number of Sponsor-packaged, labeled bottles to allow for 28 days of dosing until the next scheduled visit.

Patients will be given a dosing diary to be used for each 28-day dosing period. They should record relevant information regarding their study drug in the diary (e.g., confirmation that each daily dose was taken, reasons for missed doses).

Treatment compliance will be assessed based on return of unused drug and the dosing diary (see Section 9.9).

Patients should be instructed to take their daily dose at approximately the same times each day except for dosing on in-clinic visiting days.

Patients who undergo extensive PK/PD sampling during the Core Period (see Appendix 15.1, Table 7) should be instructed from Week 3 on to bring the AM dose with them for in-clinic visits and to ingest the dose following PK/PD blood draws.

Patients receiving limited PK/PD sampling during the Core Period (see Appendix 15.1, Table 8) should be instructed to bring the AM dose with them for all in-clinic visits and to take the AM dose following PK/PD blood draws.

Patients receiving extensive PK/PD sampling on Day 1 and 15 will also have limited PK/PD on other visit days. As a general rule, regardless of extensive or limited schedule, patients will bring in the AM dose for all visits and take this dose following PK/PD blood draws.

A minimum of 10 hours between the AM and PM dose will be required on those dosing days. Each dose should be taken with a glass of water and consumed over as short a time as possible. AG-348 may be taken with or without food. Patients should be instructed to swallow capsules whole and to not chew the capsules. For patients who have difficulty swallowing tablet(s), the Medical Monitor should be contacted to discuss administration.

Patients will receive their first dose of AG-348 in the clinic on Day 1 and then may take the remaining doses on an outpatient basis (except for in-clinic visit days, as described above).

## 9.7. Criteria for Dose Escalation, Dose Modification, or Discontinuation of Study Drug

No intra-patient dose escalations will be permitted in this study unless the DRT decides to re-assign patients' doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the patients in the terminated arm will remain in their originally assigned arm; i.e., they will not count towards the enrollment quota of the arm whose dose and schedule is being adopted. All dosing modifications, as outlined below, will be implemented following discussions with the Medical Monitor. The same criteria for dose modifications or discontinuation of study drug apply in the Extension Period as in the Core Period.

### 9.7.1. Dose Modification for Safety

The Investigator will monitor all patients for safety and tolerability. Modification of the patient's dose of AG-348 will be based on AEs and observed changes in Hb levels (see Section 9.7.2).

**Table 1: Dose Modification for Adverse Events Deemed at Least Possibly Related to AG-348**

| Adverse Events(s) | AG-348 Dose Adjustment |
|---|---|
| Grade 1 | None required. |
| Grade 2 | None required; Investigator and Medical Monitor judgment to manage as for Grade 3. |
| Grade 3 | Suspend dosing;<br>If event resolves to Grade 1 or baseline within approximately 14 days of suspension, resume dosing with 1 dose level reduction (see Table 2 below).<br>If event does not resolve to Grade 1 or baseline within approximately 14 days of suspension, permanently discontinue dosing, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor. |
| Grade 4 | Permanently discontinue dosing, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor. |

Dose modifications for Grade 2 and 3 AEs should be maintained long enough for the Investigator to be confident that the reduced dose is being well tolerated before considering a request of the Medical Monitor to permit subsequent re-escalation to the former dose level. Dosing for an individual patient will be discontinued permanently for Grade 3 and Grade 4 AEs that do not resolve to Grade 1 or baseline within approximately 14 days of suspension of dosing, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor. No patients may be re-escalated to their former dose level after a dose modification without discussion with the Medical Monitor. If following the first dose reduction for a Grade 3 AE the patient experiences a second occurrence of the same Grade 3 AE, then treatment with AG-348 must be immediately and permanently discontinued.

It should be noted that if the initial dose of 300 mg BID selected for Arm 1 demonstrates an unacceptable safety profile resulting in multiple patients undergoing dose modifications, the DRT may exercise its option to re-assign these patients' dose and schedule to match the dose and schedule of another study arm (for example, Arm 2 of the study, or to match the dose and schedule of a [potential] Arm 3, if implemented).

### 9.7.2. Dose Modification for Increase in Hemoglobin Level

It is presently unknown to what magnitude, how rapidly, or even whether, AG-348 will result in increased Hb levels in patients with PK deficiency across the variety of potential PKR mutations that may be encountered during this study. Moreover, it is unknown whether any potential increases in Hb experienced by patients in this study may be a safety risk. As a conservative measure, this study incorporates guidance for dose modification based on potential large increases in Hb levels that exceed the midpoint of the typical normal range by gender. The Investigator will monitor all patients for changes in Hb levels and should adjust the dose of AG-348 as outlined in Table 2 per the following guidelines and with Medical Monitor approval.

- Males: If Hb > 15 g/dL and confirmed with a second test, suspend dosing until Hb ≤ 13.5 g/dL; then resume dosing with a 1 dose level reduction.
- Females: If Hb > 13.5 g/dL and confirmed with a second test, suspend dosing until Hb ≤ 12.5 g/dL; then resume with a 1 dose level reduction.
- The treating Investigator will discuss with the Medical Monitor questions relating to dose modifications on an as needed basis.

**Table 2: Dose Reduction Table (by Dosing Arm)**

| Dose Group | Starting Dose | 1st Dose Reduction | 2nd Dose Reduction |
|---|---|---|---|
| Arm 1 | 300 mg BID | 200 mg BID | 100 mg BID |
| Arm 2 | 50 mg BID | 25 mg BID | TBD[1] |
| Potential Arm 3 | TBD | To approximately 50-66% of initial dose | To approximately 25-33% of initial dose |

[1] Dose to be determined by Medical Monitor.

Hemoglobin levels above the ULN by gender should be reported as an AE, graded per the CTCAEv4.03, according to the guidance provided in Section 11.2.

### 9.7.3. Stopping Criteria

Dosing for an individual patient will be discontinued permanently for Grade 3 AEs that do not resolve to Grade 1 or baseline within approximately 14 days of suspension of dosing, and for Grade 4 AEs, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor (Section 9.7.1). Other reasons for treatment termination are provided in Section 8.5.

## 9.8. Duration of Patient Participation

The duration of treatment for all patients will be up to 24 weeks in the Core Period. Patients who safely tolerate AG-348 and for whom the Investigator agrees with continuation of AG-348 treatment may be eligible to immediately roll over to the Extension Period for continued treatment (up to 2 years following completion of the Core Period).

## 9.9. Treatment Compliance

During in-clinic visits, doses of AG-348 will be ingested by the patient under the supervision of clinical facility personnel. For at-home dosing, patients will be given a dosing diary to be used for the duration of the 24-week Core Treatment Period; the diary will also be used by patients who roll over to the Extension Period. Patients should record relevant information regarding their study drug in the diary (e.g., confirmation that each daily dose was taken, reasons for missed doses) and return the diary at each study visit.

## 9.10. Study Drug Accountability

Accountability for the study drug at the clinical facility is the responsibility of the Investigator. The Investigator will ensure that the study drug is used only in accordance with this protocol.

Where allowed, the Investigator may choose to assign drug accountability responsibilities to a pharmacist or other appropriate individual.

The Investigator or delegate will maintain accurate drug accountability records indicating the drug's delivery date to the site, inventory at the site, use by each patient, and return to Agios or its designee (or disposal of the drug, if approved by Agios). These records will adequately document that the patients were provided the doses as specified in the protocol and should reconcile all study drug received from Agios. Accountability records will include dates, quantities, batch/serial numbers, expiration dates (if applicable), and patient numbers. An unblinded monitor will review drug accountability at the site on a schedule agreed to by the Sponsor.

Study drug must not be used for any purpose other than the present study.

All unused and used study drug will be retained at the site until it is inventoried by the Study Monitor after database lock has occurred. All used, unused or expired study drug will be returned to Agios or its designee or, if authorized, disposed of at the study site per the site's Standard Operating Procedures (SOPs) and documented. All material containing AG-348 will be treated and disposed of as hazardous waste in accordance with governing regulations.

## 9.11. Prior and Concomitant Medications and Treatments

### 9.11.1. Prior Medications and Procedures

All medications administered and procedures conducted within 28 days prior to the first day of study drug administration are to be recorded on the source documentation and included in the eCRF.

### 9.11.2. Prohibited Concomitant Therapy

All concomitant medications and procedures administered from 28 days before administration of study drug through the last Follow-up Visit must be recorded in the appropriate section of the source documentation and eCRF along with dosage information, dates of administration, and reason for use.

The following are prohibited at all times during participation in this study:

- Investigational drugs must be discontinued 28 days prior to the first dose of study drug;
- Products known to strongly inhibit CYP3A4 metabolism (listed in Appendix 15.4, Table 9) must be discontinued within 5 days prior to Day 1 dosing;
- Products known to strongly induce CYP3A4 metabolism (listed in Appendix 15.4, Table 10) must be discontinued within 28 days prior to Day 1 dosing;
- Products known to strongly inhibit P-gp transporter (listed in Appendix 15.4, Table 11) must be discontinued within 5 days prior to Day 1 dosing;
- Digoxin must be discontinued within 5 days prior to Day 1 dosing;
- Hematopoietic stimulating agents (EPOs, granulocyte colony stimulating factors, thrombopoietins, etc) must be discontinued no less than 28 days prior to the first dose

of study drug. [Folic acid 1 mg orally per day is required for all patients. B12 injections are permitted for patients with a prior diagnosis of B12 deficiency syndromes, but must be repleted to stability of the Hb and mean corpuscular volume (MCV) prior to enrollment in the study];

- Anabolic steroids, including testosterone preparations, administered for anemia must be discontinued no less than 28 days prior to the first dose of study drug;
- As the target population for this study consists of transfusion independent patients and transfusion of blood products could confound key endpoints of the study, blood transfusions of any type must be strictly avoided except in cases of compelling medical need. If medical circumstances permit, the Medical Monitor should be contacted for discussion before any transfusions are administered.

Drugs that displace unconjugated bilirubin from albumin (including some common sulfa antibiotics [including sulfamethoxazole/trimethoprim], cephalosporins, salicylates, and aminophylline) should be used with caution with the understanding that patients with elevated levels of unconjugated bilirubin may potentially be at risk for kernicterus syndrome (Strauss, et al. 2006).

**Potential Drug Interactions**

Strong inhibitors of CYP3A4 (listed in Appendix 15.4, Table 9) are not permitted for use with AG-348. Based on modeling of AG-348 metabolism and tolerability data from the Phase 1 MAD study, moderate inhibitors of CYP3A4 do not appear to pose a risk to patients dosed with AG-348.

In-vivo data from the Phase 1 MAD show AG-348 induces CYP3A4 and its own metabolism, presumably via CYP3A4. The use of strong CYP3A4 inducers in combination with AG-348 is expected to reduce AG-348's efficacy. Therefore, administration of strong CYP3A4 inducers (listed in Appendix 15.4, Table 10) is not permitted with AG-348.

Strong inhibitors of drug transport (listed in Appendix 15.4, Table 11) are not permitted for use with AG-348.

Digoxin is not permitted for use with AG-348.

Induction of CYP3A4 by AG-348 is expected to reduce the efficacy of certain sensitive co-medications. Co-administration of AG-348 with the drugs in Appendix 15.4, Table 12 should be done with caution, as their efficacy may be reduced.

Of note, women in the trial utilizing oral contraception must utilize barrier methods as per the Inclusion Criteria 14 (Section 8.2) while taking AG-348.

Short-term (≤ 14 days at a time, and ≤ 28 days total during the 24 week treatment period) use of topical, inhaled, intra-nasal, and systemic glucocorticoids is permitted for acute medical indications. Every effort should be made to minimize total duration of glucocorticoid therapy and utilize alternative treatments. Patients must be off glucocorticoids for at least 28 days prior to Day 1 of AG-348 dosing as per Exclusion Criterion #11 (Section 8.3). For patients who require chronic inhaled glucocorticoid therapy, Investigators should confer with the Medical Monitor for additional guidance.

The expected patient co-medications deferoxamine, deferasirox, deferiprone, and oral penicillin are not expected to interact with AG-348.

### 9.11.3. Allowed Concomitant Therapy

Medications and treatments other than those specified above are permitted during the study. All intercurrent medical conditions will be treated at the discretion of the Investigator according to acceptable local standards of medical care. Subjects may receive analgesics, antiemetics, anti-infectives (including penicillins), and antipyretics as medically indicated and consistent with the guidance in the previous two sections. Patients may continue iron chelation therapy with deferoxamine, deferasirox, or deferiprone. Patients must continue taking at least 1 mg of folic acid for the duration of the study.

All concomitant medications, including transfusions of blood products, procedures performed during the study, including those used to treat AEs, will be recorded on the eCRF.

### 9.11.4. Potential for Phototoxicity

AG-348 may cause sensitivity to direct and indirect sunlight. Patients should be warned to avoid direct sun exposure. When exposure to sunlight is anticipated for longer than 15 minutes, the patient should be instructed to apply factor 30 or higher sunscreen to exposed areas and wear protective clothing and sunglasses.

### 9.11.5. Management of Nausea, Vomiting, and Diarrhea

As the primary objective of this study is to assess the safety and tolerability of up to 24 weeks of AG-348, routine use of prophylactic anti-emetic and antidiarrheal or other classes of medications is prohibited. However, if patients experience AEs relating to nausea, vomiting, or diarrhea these may be treated at the Investigator's clinical discretion with recommended medications as follows:

- Nausea/vomiting: allowed agents include standard clinical dosing with palonosetron (Aloxi), dolasetron (Anzemet), or promethazine (Phenergen). Promethazine is a substrate for CYP2B6, and it is presently unknown if the potential for 2B6 induction after AG-348 dosing could be sufficient to reduce the therapeutic effect of promethazine. Not recommended are aprepitant (Emend; CYP3A4 inhibitor), ondansetron (Zofran; CYP3A4 inducer), chlorpromazine (Thorazine; CYP3A4 inducer), prochlorperazine (Compazine; CYP3A4 substrate, QT prolongation has been reported), and granisetron (Kytril; CYP3A4 substrate, QT prolongation has been reported);
- Diarrhea: recommended management includes standard clinical dosing with Kaopectate or other non-absorbable anti-diarrheals, diphenoxylate/atropine (Lomotil), or loperamide (Imodium). Loperamide is the least preferred choice because it is both a substrate and inhibitor for CYP3A4, a substrate for CYP2B6, and a substrate for P-gp.
- For the use of any medications not specifically mentioned above the Investigator may confer with the Sponsor’s Medical Monitor.

### 9.11.6. Other Restrictions and Precautions

Patients should be advised to refrain from altering their normal exercise routine for the first 28 days of treatment with AG-348 as symptoms of anemia could theoretically temporarily worsen before the eventual potential beneficial effect of AG-348 on the Hb level becomes evident. The elevated blood levels of 2,3-DPG commonly seen in patients with PK deficiency may produce a right shift in the Hb-O2 dissociation curve. The effect of this is to enhance oxygen delivery at the tissue level, thus counteracting to some extent the physiologic consequences of the anemia. AG-348 was shown to produce rapid decreases (within the first 12 hours following a single dose) in 2,3-DPG in two prior clinical trials with healthy adult male and female volunteers. In patients with PK deficiency who have elevated 2,3-DPG levels, it is theoretically possible that decreases in 2,3-DPG that precede correction of the anemia could result in a temporary decrease of oxygen delivery at the tissue level resulting in clinical symptoms (e.g., increased fatigue).

As discussed in Section 5.2.1.1 of this protocol and in the Investigator Brochure, AG-348 has been identified as a histamine H3 receptor antagonist/inverse agonist. No effects of histamine H3 modulation have been observed in safety pharmacology or toxicology studies. Nonetheless, patients should be monitored for potential AEs related to wakefulness and insomnia (Schwartz 2011).

# 10. STUDY ASSESSMENTS

## 10.1. Schedule of Assessments

The Schedules of Assessments for this study are provided in Appendix 15.1.

After obtaining written informed consent, patients will undergo screening evaluations. The Screening visit is to be conducted within approximately 28 days prior to first dose of study treatment.

During the Core Treatment Period, patients will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12) and monthly through Week 24 (Weeks 16, 20, and 24). Patients who safely tolerate AG-348 through Week 24 (Core Period) and for whom the Investigator agrees with continuation of AG-348 treatment may be eligible to immediately enter the Extension Period for continued treatment upon agreement of the treating Investigator and the Medical Monitor or Responsible Medical Officer. Study visits for safety and clinical activity assessments will occur approximately every 3 months during the Extension Period. All patients will undergo a follow-up assessment 4 weeks after the last dose of AG-348, regardless of whether this was due to early discontinuation, the last dose in the Core Period for a patient who chooses not to continue in the Extension Period, or the last dose of the Extension Period.

Although *not* encouraged, as a convenience for patients who travel long distances to the study site, in-clinic visits on Day 8 and Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis. For details, please refer to Table 5 in Appendix 15.1 : Schedule of Assessments. For patients having their Day 8 and/or Day 22 visits performed by the primary care physician, the principal investigator will him or herself, or have a qualified research nurse or other designated site staff member, make telephone contact with the patient to inquire about any AEs. These must be recorded as if the patient appeared in the main study center. The Principal Investigator will exercise prudent clinical judgment in determining any clinical course of action to take based on any AEs discovered. These telephone contacts on Day 8 and Day 22 must be explained to the patient in advance and scheduled in advance to maximize the likelihood of successfully making contact. Having in-clinic visits on Days 8 and/or Day 22 performed by the primary care physician will necessitate re-scheduling certain assessments that the primary care physician's office may not be reasonably expected to perform. Table 3 summarizes the details of the re-scheduling of these assessments as described in Table 5 in Appendix 15.1.

During the Extension Period, all scheduled visits must be conducted by the Investigator and at the participating authorized investigative site; local primary care visits will not be allowed.

**Table 3: Summary of Assessments When Day 8 and/or Day 22 In-Clinic Visits Are Performed By Primary Care Physician (Core Period)**

| Day 8 Visit by Primary Care Physician | | | | Day 22 Visit by Primary Care Physician | | | |
|---|---|---|---|---|---|---|---|
| Primary Care Office | Main Study Site | Assessment to Move to Day 15 Main Study Site | Assessments Not Required | Primary Care Office | Main Study Site | Assessment to Move to Day 43 Main Study Site | Assessments Not Required |
| Hematology to central lab | Phone contact with patient | 12-lead ECG | VS; serum chemistry | Hematology sample to central lab | Phone contact with patient | 12-lead ECG | VS; serum chemistry; coagulation, haptoglobin, EPO level, carboxyhemoglobin, PK/PD |

Abbreviations: ECG = electrocardiogram; EPO = erythropoietin; PK/PD = pharmacokinetics/pharmacodynamics; VS = vital signs

Whenever more than one assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (e.g., VS first, ECG, blood draw). The timing of these assessments should allow the PK blood draw to occur at the exact nominal time. The order of procedures may be revised with prior discussion between Sponsor and Site.

Minor adjustments to the timing, number of planned safety monitoring procedures (e.g., VS, ECG, blood draw), and PK/PD assessments may be made during the course of the study based on collected data to ensure appropriate safety monitoring and will not require a protocol amendment. These minor changes will require prior approval from the Sponsor's Medical Monitor (or Responsible Medical Officer) as well as appropriate documentation in the study records. The addition of new safety monitoring procedures or other assessments will require a protocol amendment.

## 10.2. Informed Consent and Confirmation of Eligibility

A complete description of the study is to be presented to each potential patient and a signed and dated informed consent is to be obtained before any study specific procedures are performed. Separate informed consent forms will be used for the Core and Extension Periods.

Patient's eligibility will be confirmed at Screening and within 24 hours prior to study treatment dose. If a patient is determined to be ineligible, the patient will be excluded from participation.

## 10.3. Demographic Data, Medical and Medication History

Patient demographic data, including gender, date of birth, age, race, and ethnicity, will be obtained at Screening. Collection of demographic data will be modified by country regulatory requirements, as appropriate.

Medication history, including all relevant prior medical history and current medical conditions, will be obtained at the Screening assessment and on Day -1; all concomitant medications administered and procedures conducted within 28 days prior to Day 1 should be reported in the source documentation and eCRF. Investigators will be asked to provide information on the patient's history of any medical diagnoses (e.g., iron overload) and surgical procedures (e.g., splenectomy, cholecystectomy) pertaining to their diagnosis of PK deficiency and prior available

complete blood counts (CBCs) over the preceding 6 months and transfusion history over the preceding 12 months prior to the date of signing informed consent.

## 10.4. PKR Enzymatic Assay and PKR Genotyping

Assessments for PKR enzymatic activity and PKR genotyping will be performed at Screening only for confirmation of study eligibility. PKR enzymatic assays will be conducted at [redacted] PKR genotyping will be conducted at [redacted]

## 10.5. Safety Assessments

### 10.5.1. Physical Examination, Height, and Weight

A complete physical examination (including neurological examination; genital and rectal examinations will be performed at the discretion of the Investigator) will be obtained at Screening, Baseline, Week 12, and the Follow up Visit (Week 28). The neurological examination must include an assessment of general orientation and mental status including level of alertness (assess as normal or abnormal and specify any abnormality). Limited focused physical examinations will be performed at all other visits during the Core Period. Focused physical examinations, including neurological examination, will continue every 3 months during the Extension Period; a complete physical examination will be performed at Month 30. Any findings will be recorded on the eCRF. Height will be collected at Screening only.

### 10.5.2. Vital Signs

Vital signs, including systolic and diastolic BP, heart rate, respiratory rate, and temperature, will be obtained according to the according to the Schedules of Assessments (Appendix 15.1). Vital sign measurements will be repeated if clinically significant or machine/equipment errors occur. Out of range BP or heart rate measurements will be repeated at the Investigator's discretion. Any confirmed, clinically significant vital sign measurements will be recorded as AEs.

### 10.5.3. Electrocardiogram

A 12-lead ECG will be obtained according to the Schedules of Assessments (Appendix 15.1). The ECGs will be measured using an ECG machine that calculates the heart rate and measures the portion of the ECG wave from the beginning of the P wave to the beginning of the QRS interval on an electrocardiogram (QRS) complex (PR), QRS, QT, QTcB (Bazett correction formula), and QTcF. Only QTcF (not QTcB) will be used for determination of eligibility.

The 12-lead ECGs should be obtained following 5 minutes of recumbency. The Screening ECG will be performed at least 7 days prior to Day 1 dosing. ECGs will be repeated if clinically significant abnormalities are observed, if artifacts are present, or if machine/equipment errors occur. Any confirmed, clinically significant ECG findings will be recorded as AEs.

### 10.5.4. DXA Scans

DXA scans (hip and spine) will be performed at Screening to obtain T and Z scores and bone mineral density that will serve as a baseline measure for all enrolling patients. An additional DXA scan for the Core Period will be conducted in the interval between Week 24 and Week 28,

and scans will be conducted at Months 18 and 30 during the Extension Period as indicated in the Schedules of Assessments (Appendix 15.1). All redacted DXA scan reports must be held at the study site and will be collected by the Sponsor.

### 10.5.5. Safety Laboratory Assessments

#### 10.5.5.1. Hematology, Serum Chemistry, Coagulation Studies, and Urinalysis

Laboratory values obtained prior to Screening and RBC antibodies obtained at Screening will be performed at a local laboratory. On-study clinical laboratory evaluations are to be performed by a central laboratory. If Investigators believe that it is clinically indicated to obtain safety laboratory results from their own local laboratories on the day of the subject's visit, and before the results are returned from the designated central laboratory, they are free to exercise their discretion to do so. Investigators should be aware that since the Hb level is a secondary endpoint of the study, it behooves them to minimize blood volumes drawn, and samples for the central lab must still be collected, since it will serve as the official lab result for this study.

Clinical laboratory evaluations are to be collected according to the Schedules of Assessments (Appendix 15.1). In addition, all clinically significant laboratory abnormalities noted on testing will be followed by repeat testing and further investigated according to the judgment of the Investigator. Please note that serum estradiol, free and total testosterone, and CBC will be collected in the AM at any 2 time points during Screening at least 2 days apart in addition to Baseline/Day 1 (total of 3 time points prior to Day 1 dosing).

The following safety laboratory parameters are to be determined:

| | |
|---|---|
| **Hematology**: | HCT, Hb, RBC count, absolute reticulocyte count, percent reticulocyte count, MCV, mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count with automated (or manual, as indicated) differential, ANC, and absolute lymphocyte count (ALC), and platelet count. In the event that the designated central laboratory for the study is unable to provide a valid result for any specific component of the defined CBC for a specific patient, the site may be asked to have the test performed at their local laboratory. The result of the local CBC will be entered into the study database along with the local normal reference range. G6PD and RBC antibody screen will be performed at Screening only |
| **Other** | EPO, Hp, COHb, 25-hydroxy vitamin D2 and D3. |
| **Serum Chemistry:** | alkaline phosphatase (ALP), sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide ($CO_2$) or bicarbonate, albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, LDH, ALT, AST, total bilirubin, indirect bilirubin, (and estimated creatinine clearance or GFR for screening only, as appropriate). |

| | |
|---|---|
| **Sex Hormones**: | testosterone (total and free), estrone, and estradiol. FSH will only be performed at Screening for female patients only for confirmation of post-menopausal status. |
| **Bone Turnover** | serum osteocalcin-N-mid and CTX. |
| **Lipids** | total cholesterol, HDL-C, triglycerides. |
| **Iron Panel** | iron, total iron-binding capacity (TIBC), transferrin saturation, ferritin. |
| **Coagulation Studies:** | fibrinogen, aPTT, INR. |
| **Urinalysis**: | color and appearance, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, pregnancy screen (dipstick), occult blood. Microscopic inspection of sediment will only be performed for cause or to investigate an abnormal dipstick finding per the Investigator's discretion. |

#### 10.5.5.2. Screening Serology

A blood sample for serology, including HBsAg, HCV Ab screen, and HIV1 and HIV2 Ab, is to be collected from all patients at Screening.

### 10.5.6. Menstrual Cycle Diary

Menstruating female patients will be required to fill out a paper-based menstrual cycle diary each month in order to monitor any changes. Diaries will be dispensed and collected as indicated in the Schedules of Assessments (Appendix 15.1). Patients will record the start date, stop date, and any notable characteristics of each menstrual cycle.

### 10.5.7. Adverse Events

Each patient will be carefully monitored for the development of any AEs throughout the study from signing of the informed consent through all scheduled study follow-up visits, or withdrawal of consent, whichever occurs first. In addition, SAEs that are assessed as possibly or probably related to study treatment that occur > 30 days post-treatment also are to be reported.

Adverse events will be evaluated by the Investigator and recorded as described in the Schedules of Assessments. On dosing visits, all AEs (elicited and spontaneously reported) will be continuously evaluated by the Investigator and recorded. At any non-dosing day visit, AEs will be evaluated by the Investigator and recorded. Any AEs already documented at a previous assessment and designated as ongoing will be reviewed at subsequent visits or assessment time points as necessary. If these AEs have resolved, this will be documented.

All AEs will be graded using the CTCAE grading system (Appendix 15.3 ).

Complete details on AE monitoring are provided in Section 11.

## 10.6. Pharmacokinetic Assessments

The first approximately 10 patients treated in the Core Period, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 15.1, Table 7. The remainder of treated patients will undergo limited PK sampling as detailed in Appendix 15.1, Table 8. The in-clinic visit on Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis. In this instance, PK sampling will not be required on Day 22. (Additional details regarding Day 8 and Day 22 visits performed by the patient's primary care physician can be found in Table 5 in Appendix 15.1: Schedule of Assessments: Core Period.) During the Extension Period, predose PK samples will be drawn for the measurement of trough levels of AG-348 and AGI-8702 at each study visit (every 3 months; see Appendix 15.1, Table 6).

The collection times for post-dose PK samples will start from the time that dosing is completed. (For example, a PK draw at 30 minutes will be collected 30 minutes after the last capsule has been ingested.) The completion time of each dose will be recorded.

Procedures for sample collection and processing will be provided in a separate study manual. The actual time point of each sample collection will be recorded.

Samples for PK and PD assessments may be retained for up to 2 years from collection.

## 10.7. Pharmacodynamic Assessments

The first approximately 10 patients treated in the Core Period, contingent on clinical site feasibility, will undergo extensive PD sampling for 2,3-DPG and ATP as detailed in Appendix 15.1, Table 7. The remainder of treated patients will undergo limited PD for 2,3-DPG and ATP sampling as detailed in Appendix 15.1, Table 8. During the Extension Period, predose PD samples will be drawn for the measurement of trough levels of 2,3-DPG, ATP, [redacted] at each study visit (every 3 months; see Appendix 15.1, Table 6).

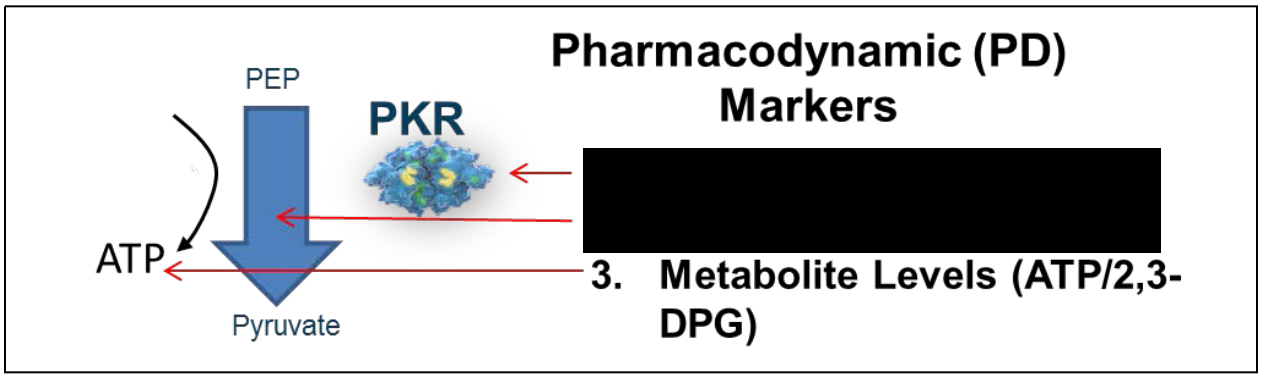
The collection times for post-dose PD samples will start from the time that dosing is completed. (For example, a PD draw at 30 minutes will be collected 30 minutes after the last capsule has been ingested.) The completion time of each dose will be recorded.

Procedures for sample collection and processing will be provided in a separate study manual. The actual time point of each sample collection will be recorded.

Pharmacodynamic assessments during the Core Period will include 2,3-DPG, ATP, [redacted] The in-clinic visit on Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis. In this instance, PD sampling will not be required on Day 22. (Additional details regarding Day 8 and Day 22 visits performed by the patient's primary care physician are stated in Table 5 in Appendix 15.1: Schedule of Assessments.)

Figure 4 provides a brief schematic outlining the PKR reaction and how each of these PD assessments fits into a complete mechanistic understanding of the action of AG-348.

**Figure 4: PKR Enzymatic Reaction**




The PKR enzyme catalyzes the PEP to pyruvate reaction, with concomitant formation of ATP.

- Binding of AG-348 to the PKR tetramer can be assessed through an ex-vivo biochemical assay of cell lysates from AG-348 treated patients. Because WBCs contain a high level of pyruvate kinase from a non-PKR pyruvate kinase isoform, WBCs are first removed by filtration before the purified red cells are frozen. [redacted] It has been reported in the literature that there may be compensatory expression of PKM2 in the RBCs of some patients with PKR deficiency (Black, et al. 1979; Kahn, et al. 1975; Rijksen, et al. 1990). Therefore, levels of PKM2 and appropriate reference proteins (e.g. actin, GAPDH) may be evaluated in these whole blood samples.
- [redacted]
- AG-348 target engagement [redacted] has been shown in preclinical models and healthy volunteer clinical studies to result in accumulation of ATP and depletion of the upstream metabolite 2,3-DPG. Therefore, levels of these metabolites will be measured by mass spectrometry in frozen whole blood samples.

The first approximately 10 patients treated during the Core Period, contingent on clinical site feasibility, will undergo extensive PD sampling as detailed in Appendix 15.1, Table 7. The remainder of treated patients will undergo limited PD sampling as detailed in Appendix 15.1, Table 8. The collection times for post-dose PD samples will start from the time that dosing is

completed. (For example, a PD draw at 30 minutes will be collected 30 minutes after the last capsule has been ingested.) The completion time of each dose will be recorded.

Blood samples will be stored at the site and regularly transported at -80°C ±10 C to the bioanalytical laboratory for analysis. Procedures for sample collection and processing will be provided in a separate study manual.

## 10.8. Ordering of Blood Sample Collection

When more than 1 blood sample is collected at the same nominal time, the samples will be collected in the following order:

1. Safety laboratory assessments
2. PK
3. PD (2,3 DPG, ATP)

## 10.9. Sample Processing, Storage, and Shipment

Instructions for the processing, storage and shipment of all study samples for central analysis will be provided in a separate study manual.

# 11. ADVERSE EVENTS

Monitoring of AEs will be conducted throughout the study. Adverse events and SAEs will be recorded in the source documentation and eCRF from time of the signing informed consent through the Follow-up Visits for randomized patients. All AEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

All SAEs will be followed through 30 days after the last dose of study treatment or until the SAE has resolved. Any SAEs that are assessed as possibly or probably related to study treatment that occur > 30 days post-treatment also are to be reported.

## 11.1. Definition of Adverse Events

### 11.1.1. Adverse Event

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An AE can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

### 11.1.2. Suspected Adverse Reaction

A suspected adverse reaction is any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of expedited safety reporting, 'reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the AE.

### 11.1.3. Unexpected Adverse Event

An unexpected AE is one for which the nature or severity of the event is not consistent with the applicable product information, e.g., the Investigator's Brochure.

### 11.1.4. Serious Adverse Event

An AE or suspected adverse reaction is considered serious (SAE) if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death;
- Life-threatening. Life-threatening means that the patient was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form;
- In-patient hospitalization or prolongation of existing hospitalization. Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered AEs if the illness or disease existed before the patient was enrolled in the study, provided that it did not deteriorate in an unexpected manner during the study (e.g., surgery performed earlier than planned);

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- Congenital anomaly/birth defect;
- Important medical event. An important medical event is an event that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the patient or patient and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions for SAEs. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalization, or the development of drug dependency or drug abuse.

#### 11.1.4.1. Potential Severe Drug-Induced Liver Injury

The document entitled FDA Guidance for Industry, Drug-Induced Liver Injury: Premarketing Clinical Evaluation (FDA 2009) provides guidance on how the measurement of various laboratory parameters may be used to assess a given drug's potential to cause severe liver injury (i.e., irreversible liver failure that is fatal or requires liver transplantation). Such cases are suggested by the following three components:

1. The drug causes hepatocellular injury, generally shown by a higher incidence of 3-fold or greater elevations above the ULN of ALT or AST than the (non-hepatotoxic) control drug or placebo;
2. Among trial patients showing such aminotransferase elevations, often with aminotransferases much greater than 3×ULN, one or more also show elevation of serum total bilirubin to > 2×ULN, without initial findings of cholestasis (elevated serum ALP);
3. No other reason can be found to explain the combination of increased aminotransferases and total bilirubin, such as viral hepatitis A, B, or C; pre-existing acute liver disease; or another drug capable of causing the observed injury.

Clinical safety laboratory results compatible with the definition of drug-induced liver injury (DILI) stated above must be repeated for confirmation as soon as possible, and if confirmed, will be scored as an unacceptable AE and reported to FDA as a serious unexpected AE.

## 11.2. Procedures for Reporting Adverse Events and Serious Adverse Events

Each patient must be carefully monitored for the development of any AEs. This information should be obtained in the form of non-leading questions (e.g., "How are you feeling?") and from signs and symptoms detected during each examination, observations of study personnel, and spontaneous reports from patients.

All AEs (serious and non-serious) spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures will be recorded in the source documentation and eCRF. Any clinically relevant deterioration in laboratory assessments or other clinical findings is considered an AE and must be recorded in the appropriate source documentation and eCRF. Treatment-emergent

abnormal clinical laboratory results should generally be reported as AEs if there are accompanying symptoms; if additional diagnostic evaluations or medical (including drug therapy) or surgical interventions are undertaken; if a change in study drug dosing or study drug discontinuation is required; or, if the laboratory result is considered clinically significant by the Investigator. Although it is an objective of this study to determine if treatment with AG-348 results in increased Hb levels in patients with PK deficiency, overshoot of the Hb level above the ULN by gender is not recommended and should be reported as an AE, graded per the CTCAEv4.03. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

All SAEs that occur during the course of the study must be promptly reported by the Investigator to [redacted] Global Safety and Pharmacovigilance (see below). Deaths and AEs assessed as life-threatening are to be reported immediately and SAEs that meet other criteria are to be reported within 24 hours from the point in time when the Investigator becomes aware of the SAE. All SAEs must be reported whether or not they are considered causally related to AG-348. Serious AE forms will be completed and the information collected will include subject number, a narrative description of the event, and an assessment by the Investigator as to the severity of the event and relatedness to study drug. Follow-up information on the SAE may be requested by the Sponsor or Medical Monitor.

**Contact Information:**

[redacted] **Safety Management**

SAE Hotline (United States): [redacted]
SAE Fax (United States): [redacted]

SAE Hotline (France): [redacted]
SAE Fax (France): [redacted]

SAE Hotline (Netherlands): [redacted]
SAE Fax (Netherlands): [redacted]

SAE Hotline (Italy): [redacted]
SAE Fax (Italy): [redacted]

SAE Hotline (United Kingdom): [redacted]
SAE Fax (United Kingdom): [redacted]

E-mail: [redacted]

If there are serious, unexpected adverse drug reactions associated with the use of AG-348, the Sponsor will notify the appropriate regulatory agency(ies) and all participating Investigators on an expedited basis. The local IRB/IEC will be promptly notified based on local regulations where required by the IRB/IEC of all serious, unexpected adverse drug reactions involving risk to human subjects.

All AEs, whether serious or not, will be described in the source documents in the database. All new events, as well as those that worsen in intensity or frequency relative to baseline, which occur after signing the informed consent through the final Follow-up Visit (Day 29 ± 3 days) must be recorded. Adverse events that are ongoing at the time of treatment discontinuation

should be followed up to 30 days after the last dose of study treatment. All SAEs will be followed up to 30 days after the last dose of study treatment, or until the SAE has resolved.

Information to be reported in the description of each AE includes:

- A medical diagnosis of the event (if a medical diagnosis cannot be determined, a description of each sign or symptom characterizing the event should be recorded);
- The date of onset of the event;
- The date of resolution of the event;
- Whether the event is serious or not;
- Intensity of the event (see below for definitions);
- Relationship of the event to study treatment (see below for definitions);
- Action taken: none; change in the study drug administration (e.g., temporary interruption in dosing); drug treatment required; non-drug treatment required; hospitalization or prolongation of hospitalization required (complete SAE page); diagnostic procedure performed; patient discontinued from the study (complete End of Study visit);
- Outcome: patient recovered without sequelae; patient recovered with sequelae; event ongoing; patient died (notify the Medical Monitor immediately, and complete the SAE form).

**Intensity** of all AEs will be graded according to the NCI CTCAE Version 4.03 (Appendix 15.1).

**Relationship** to study drug administration will be determined by the Investigator according to the following criteria:

- **Not Related**: Exposure to the study treatment did not occur, or the occurrence of the AE is not reasonably related in time, or the AE is considered unlikely to be related to the study treatment.
- **Possibly Related**: The study treatment and the AE were reasonably related in time, and the AE could be explained equally well by causes other than exposure to the study treatment. An attribution of possibly related means that there are facts in evidence to suggest a possible relationship.
- **Probably Related**: The study treatment and the AE were reasonably related in time, and the AE was more likely explained by exposure to the study treatment than by other causes, or the study treatment was the most likely cause of the AE. An attribution of probably related means that there are facts in evidence to suggest a probable relationship.

For the purpose of safety analyses related to final database review, all AEs that are classified as possible or probable will be considered treatment-related AEs.

## 11.3. Pregnancy Reporting

Pregnancy is neither an AE nor an SAE, unless a complication relating to the pregnancy occurs (e.g., spontaneous abortion, which may qualify as an SAE). However, any pregnancy in a participating female patient or a female partner of a participating male patient that occurs during this study or within 30 days following the last dose of AG-348 must be reported to the Sponsor or Medical Monitor within 30 days of being notified of the pregnancy. The Investigator must follow up and document the course and outcome of all pregnancies even if the patient was discontinued from the study or if the study has finished. The female patient or partner of a male patient should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus. Monitoring should continue until conclusion of the pregnancy.

All outcomes of pregnancy must be reported by the Investigator to the Sponsor or Sponsor's designee on a Pregnancy Outcome Report form within 30 days after he/she has gained knowledge of the delivery or elective abortion.

Any SAE that occurs during pregnancy must be recorded on the SAE report form (e.g., maternal serious complications, spontaneous or therapeutic abortion, ectopic pregnancy, stillbirth, neonatal death, congenital anomaly, or birth defect) and reported within 24 hours in accordance with the procedure for reporting SAEs.

All patients, male and female, must agree to use effective contraception during the entire study and for 30 days following the last dose of AG-348. Abstinence is an acceptable method only when this is in line with the normal life style of the patient, meaning that the patient plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence, e.g. calendar, sympathothermal and post-ovulation methods, and withdrawal are not acceptable methods of contraception.

# 12. STATISTICAL METHODS

The primary objective of this Phase 2 study is to evaluate the safety and tolerability of up to 24 weeks of AG-348 in patients with PK deficiency. Therefore, analyses will be primarily descriptive in nature; no formal hypothesis testing will be conducted. All analyses will be conducted separately within each dose arm, or pooled where appropriate. Analyses of safety and of indicators of clinical activity will be conducted for the Core Period, Extension Period, and overall, if applicable. For the Core Period, the data to be analyzed will include all collected data through 24 weeks of treatment for patients who directly enter the Extension period. For patients who do not enter the Extension Period, the analyses will include all collected data through the duration of treatment (24 weeks or less) plus 4 week follow up data. For patients who move directly from the Core to the Extension Period, the 4 week follow up data will be analyzed as part of the Extension Period.

## 12.1. Sample Size Estimation

Due to the rare disease setting, the minimal sample size in each dose arm may be determined by feasibility. In order to evaluate the primary objective of safety and tolerability of AG-348 in Arm 1 and Arm 2, up to a maximum of 25 patients may be randomized onto each arm. The actual number of patients enrolled into Arms 1 and 2 will depend on the safety reviews and decisions made by the DRT. In addition, up to 25 additional patients may be enrolled to evaluate an additional dose arm (Arm 3; see Section 7.1). As for Arms 1 and 2, the actual enrollment in a potential Arm 3 will depend on the safety reviews and decisions made by the DRT. Therefore, up to approximately 75 total patients may be enrolled in this study across 2 to 3 dose arms.

Table 4 provides the probability within a dose arm of detecting 1 or more AEs with varying sample size and the true underlying AE rates.

**Table 4: Sample Size Estimation**

| Sample Size | True Underlying AE Rate | | |
|---|---|---|---|
| | **15%** | **10%** | **5%** |
| 5 | 56% | 41% | 23% |
| 10 | 80% | 65% | 40% |
| 15 | 91% | 79% | 54% |
| 25 | 98% | 93% | 72% |

## 12.2. Populations for Analysis

The following patient populations (i.e., analysis sets) will be evaluated and used for presentation of the data:

- Safety Analysis Set: All patients who are enrolled and receive any dose of study treatment. The Safety Analysis Set will be the primary set for the analysis of safety

data. Patients will be classified according to treatment received, where treatment received is defined as the assigned treatment if it is received at least once, or as the first treatment received if assigned treatment is never received. Unless otherwise stated, the Safety Analysis Set will be the default analysis set for all data analyses.

- Pharmacokinetic Analysis Set: All patients in the Safety Analysis Set with sufficient plasma sample data to assess PK parameters. Results of the potential PD activity of AG-348 will also be based on the PK analysis set.
- Efficacy Analysis Set: All patients who are enrolled and achieve at least 50% compliance with their assigned dose intensity for at least 4 weeks of continuous dosing. Additional efficacy analyses may be performed on subsets of patients with other degrees of compliance with their assigned dose intensity and/or duration of dosing. The Efficacy Analysis Set will be the primary set for the analysis of preliminary clinical activity data. Patients will be classified according to assigned treatment.

[redacted] If such analyses are performed, they will be described in a separate PK Statistical Analysis Plan (SAP) and may be reported separately in a stand-alone report.

## 12.3. Procedures for Handling Missing, Unused, and Spurious Data

No imputation will be performed for missing data elements. When summarizing AE data, partial dates will be imputed as described in the SAP. Additional rules addressing the handling of missing data will be detailed in the SAP.

## 12.4. Interim Analysis

No formal statistical interim analysis will be conducted. Safety data will be reviewed on an ongoing basis by the DRT, who will meet to review safety, PK, PD, and preliminary clinical activity data at regular intervals (approximately every 6 weeks during the Core Period and approximately every 3 months during the Extension Period once all patients have completed the Core Period) throughout the duration of the Core Period. The DRT's decisions to suspend, terminate, or open a potential third dosing arm, or re-assign patients' dosing in a terminated arm to match the dose and schedule of another arm of the study will be based on the totality of the data including, safety, PK, PD, and preliminary clinical activity (e.g., changes in Hb levels).

Additional interim reviews of data may be conducted to support decision making concerning the current clinical study, the sponsor's development programs in general, or in case of any safety concerns.

## 12.5. Statistical Methodology

### 12.5.1. General Methods

This study will be primarily descriptive in nature; therefore, there will be no formal hypothesis testing. Summaries will be produced for disposition, baseline disease characteristics and

demographic data including genotype, safety measurements, PK, PD parameters and indicators of clinical activity. Data from each AG-348 dose group will be analyzed separately, and pooled across all dose groups where appropriate. All data will also be listed by individual patient.

Categorical variables will be summarized by frequency distributions (number and percentages) and continuous variables will be summarized by descriptive statistics (mean, standard deviation [SD], median, minimum, and maximum).

The study database will be locked and statistical analysis will be performed after all patients have completed the Core Period. The results of this analysis will be presented in a clinical study report (CSR). Additional data collected during the Extension Period after the CSR database lock will be analyzed for inclusion in a subsequent CSR addendum.

### 12.5.2. Disposition

A summary of the disposition of patients will be presented, including the number enrolled, the number treated, and the reasons for study discontinuation. Entry criteria and protocol deviations will be listed.

### 12.5.3. Exposure and Safety Analyses

Patients will receive multiple PO doses of AG-348 over a 24-week treatment period. The actual dose and duration in days of AG-348, as well as the dose intensity (computed as the ratio of actual dose received and actual duration) and the relative dose intensity (computed as the ratio of dose intensity and planned dose received/planned duration) will be listed and summarized using descriptive statistics by dose arm.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and the incidence of TEAEs (new or worsening from baseline) will be summarized by primary system organ class (SOC), preferred term, CTCAE Version 4.03 severity, outcome, action taken with study drug, and relationship to the study drug by dose group. Separate summaries will be produced for all TEAEs, treatment-related AEs (those considered by the Investigator as at least possibly drug related), SAEs, discontinuations due to AEs, and AEs ≥ Grade 3 severity. Individual patient listings will be provided for deaths, SAEs, AEs leading to interruption and/or reduction of study drug dose, and AEs leading to discontinuation of treatment.

Analyses of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined as any AE with onset beginning at the day of first administration of study drug, throughout the treatment period until 30 days after the last dose of study drug, or any event that was present at baseline but worsened in intensity or was subsequently considered drug-related by the Investigator through the end of the study.

Descriptive statistics will be provided for clinical laboratory values (e.g., hematology, serum chemistry, coagulation studies, urinalysis) and VS data, presented as both actual values and changes from baseline relative to each on-study evaluation. Shift analyses will be conducted for selected laboratory parameters based on the baseline CTCAE grade to maximum CTCAE grade. Where applicable CTCAE terms do not exist, a grading system based on the upper and/or lower limits of normal will be used to classify the results.

No formal hypothesis-testing analysis of AE incidence rates will be performed.

Electrocardiogram analyses will include individual patient listings and summaries of abnormal and clinically significant ECG results. Actual values and changes from baseline in PR, QRS, and heart-rate corrected QT interval (QTc) intervals will be summarized by visit and dose arm. Full details of the QTc analysis including correction methods used will be described in the SAP.

Data collected from the menstrual diaries such as the start and stop dates of the menses and the patient reported characteristics of the menses will be presented in a by-patient listing. Additional descriptions of the data may also be performed.

Concomitant medications will be coded using the WHO Drug Dictionary. Concomitant medications prior to and after the start of the study drug will be listed by patient, and summarized by ATC term and dose arm.

### 12.5.4. Pharmacokinetic Analyses

Descriptive statistics will be used to summarize PK parameters for the parent compound AG-348 and the metabolite AGI-8702 for each dose group, and where appropriate, for the entire population. Pharmacokinetic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum and maximum, geometric mean, and geometric coefficient of variation. PK analyses will be described in a separate PK SAP.

### 12.5.5. Pharmacodynamic Analyses

Descriptive statistics will be used to summarize PD parameters for 2,3-DPG and ATP for each dose group, and where appropriate for the entire population. Pharmacodynamic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation %. PD analyses will be described in a separate PD SAP.

### 12.5.6. Aromatase Hormone Analysis

The analyses of serum sex hormones will use appropriate graphic displays and statistical analyses to evaluate patient change from baseline for each parameter. These analyses will include summaries of actual values and change from baseline using appropriate descriptive statistics (mean, SD, median, min and max) as described above.

[redacted] These analyses will present information by each dose arm, and analyses of a pooled AG-348 cohort. Additional details regarding these analyses will be provided in the SAP.

### 12.5.7. Clinical Activity

Details on analyses to evaluate indicators of potential clinical activity of AG-348 in patients with PK deficiency will be described in the SAP. These will include changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation (serum iron/iron binding capacity). Characterization of Hb response which will include, but is not limited to percent of patients with increase in Hb, time to Hb response, and duration of Hb response will be explored.

[redacted]

## 12.6. Procedures for Reporting Deviations to Original Statistical Analysis Plan

All deviations from the original SAP will be provided in the final CSR.

# 13. ADMINSTRATIVE REQUIREMENTS

## 13.1. Good Clinical Practice

The study will be conducted in accordance with the ICH for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s). The Investigator will be thoroughly familiar with the appropriate use of the study drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study, and retained according to the appropriate regulations.

## 13.2. Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki (Appendix 15.5).

The Investigator must obtain IRB approval for the investigation and must submit written documentation of the approval to the Sponsor before he or she can enroll any patient into the study. The IRB will review all appropriate study documentation in order to safeguard the rights, safety, and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, Investigator's Brochure, informed consent, advertisements (if applicable), written information given to the patients, signed Dose Escalation Interim Safety Reports, annual progress reports, and any revisions to these documents will be provided to the IRB. The IRB is to be notified of any amendment to the protocol in accordance with local requirements. Progress reports and notifications of serious unexpected adverse drug reactions are to be provided to the IRB according to local regulations and guidelines.

## 13.3. Patient Information and Informed Consent

The Investigator or trained designee will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk, and benefit of the study. Patients must also be notified that they are free to discontinue from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

After the study has been fully explained, written informed consent will be obtained from the patient prior to study participation.

The patient's signed and dated informed consent must be obtained before conducting any study-related procedures. The Investigator must maintain the original, signed consent form. A copy of the signed form must be given to the patient.

The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

## 13.4. Patient Confidentiality

In order to maintain patient privacy, all source documents, study drug accountability records, study reports and communications will identify the patient by the assigned patient number. The

Investigator will grant monitor(s) and auditor(s) from the Sponsor or its designee and regulatory authority(ies) access to the patient's original medical records for verification of data gathered on the source documents and to audit the data collection process. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

## 13.5. Protocol Compliance

The Investigator will conduct the study in compliance with the protocol. Modifications to the protocol should not be made without agreement of both the Investigator and the Sponsor. Changes to the protocol will require written IRB approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to patients. The IRB may provide, if applicable, where regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval/favorable opinion of the IRB. The Sponsor or designee will submit all protocol modifications to the regulatory authority(ies) in accordance with the governing regulations.

When immediate deviation from the protocol is required to eliminate an immediate hazard(s) to patients, the Investigator will contact the Sponsor's Medical Monitor (or Responsible Medical Officer), if circumstances permit, to discuss the planned course of action. Any departures from the protocol must be fully documented in the source documents/database.

## 13.6. Data Management

A paper or eCRF will be completed for each patient or an electronic data capture (EDC) system will be used. The EDC system (Medidata Rave®) is a software tool designed to ensure quality assurance and facilitate data capture during clinical trials. Through a system regulated workflow that includes barcode scanning and interfaces to medical equipment to avoid manual data entry, study operations performance is controlled and captured in real time. The system is fully Code of Federal Regulations (CFR) 21 Part 11 compliant. Source documentation supporting the data should indicate participation in the study and should document the dates and details of study procedures, AEs, and patient status. The Investigator, or trained designee should complete and the Investigator should verify the source documents as the information is collected. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data. The Investigator will ensure the accuracy, completeness, and timeliness of the data reported to the Sponsor. Data collection processes and procedures will be reviewed and validated to ensure completeness, accuracy, reliability, and consistency. A complete audit trail will be maintained of all data changes. The Investigator or designee will cooperate with the Sponsor's representative(s) for the periodic review of study documents to ensure the accuracy and completeness of the data capture system at each scheduled monitoring visit. Electronic consistency checks and manual review will be used to identify any errors or inconsistencies in the data. This information will be provided to the respective study sites by means of electronic or manual queries. The Investigator or designee will prepare and maintain adequate and accurate study documents (medical records, ECGs, AE, and concomitant medication reporting, raw data collection forms, etc.) designed to record all observations and other pertinent data for each patient receiving study treatment. The Investigator will allow

Sponsor representatives, contract designees, authorized regulatory authority inspectors, and the IRB to have direct access to all documents pertaining to the study.

## 13.7. Source Document/Case Report Form Completion

Source documents will be completed for each study patient. It is the Investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the patient's source document. The source document should indicate the patient's participation in the study and should document the dates and details of study procedures, AEs and patient status.

The Investigator, or designated representative, should complete the source document as soon as possible after information is collected, preferably on the same day that a study patient is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.

The Investigator will sign and date each required assessment for all study patients. The Investigator will retain all completed source documents.

## 13.8. Direct Access to Source Data

The study will be monitored by the Sponsor or its designee. Monitoring will be done by personal visits from a representative of the Sponsor (site monitor) and will include on-site review of the source documents for completeness and clarity, cross-checking with source documents, and clarification of administrative matters will be performed. The review of medical records will be performed in a manner to ensure that patient confidentiality is maintained.

The site monitor will ensure that the investigation is conducted according to protocol design and regulatory requirements by frequent communications (letter, telephone, e-mail, and fax).

All unused study drug and other study materials are to be returned to the Sponsor or designee after the study has been completed and the database has been locked.

Regulatory authorities, the IRB, and/or the Sponsor's clinical quality assurance group or designee may request access to all source documents, database, and any other applicable study documentation for an on-site audit or inspection. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities.

## 13.9. Record Retention

The Investigator will maintain all study records according to ICH-GCP and applicable regulatory requirement(s). Records will be retained for at least 2 years after the last marketing application approval or 2 years after formal discontinuation of the clinical development of the investigational product or according to applicable regulatory requirement(s). If the Investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility. The Sponsor must be notified in writing if a custodial change occurs.

## 13.10. Liability and Insurance

The Sponsor has subscribed to an insurance policy covering, in its terms and provisions, its legal liability for injuries caused to participating persons and arising out of this research performed

strictly in accordance with the scientific protocol as well as with applicable law and professional standards.

## 13.11. Publication of Study Findings and Use of Information

All information regarding AG-348 supplied by the Sponsor or designee to the Investigator is privileged and confidential information. The Investigator agrees to use this information to accomplish the study and will not use it for other purposes without consent from the Sponsor. It is understood that there is an obligation to provide the Sponsor with complete data obtained during the study. The information obtained from the clinical study will be used towards the development of AG-348 and may be disclosed to regulatory authority(ies), other Investigators, corporate partners, or consultants as required.

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# 15. APPENDICES

## 15.1. Schedules of Assessments

### Table 5: Schedule of Assessments: Core Period

| Timing | Pre-Treatment | | Month 1 | | | | Months 2 and 3 | | | Months 4, 5 and 6 | | | Follow Up[1] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Visit | Screening | | Baseline / D1 | W1 | W2 | W3 | W6 | W9 | W12 | W16 | W20 | W24 | W28 |
| Study Day | -28 to -1 | -26 to -1[2] | 1 | 8[3] | 15 | 22[3] | 43 | 64 | 85 | 113 | 141 | 169 | 197 |
| Visit Window | | | | ± 2 D | ± 2 D | ± 2 D | ± 7 D | ± 7 D | ± 7 D | ± 7 D | ± 7 D | ± 7 D | ± 7 D |
| Written Informed Consent | X | | | | | | | | | | | | |
| PK enzyme assay (confirmation of PK deficiency) | X | | | | | | | | | | | | |
| PKR Genotype (for randomization) | X | | | | | | | | | | | | |
| UGT1A1 Genotype | X | | | | | | | | | | | | |
| Demographics | X | | | | | | | | | | | | |
| Medical/Surgical History (General and PK deficiency-specific)[4] | X | | | | | | | | | | | | |
| Medication History | X | | | | | | | | | | | | |
| Transfusion History | X | | | | | | | | | | | | |
| Confirmation of Vaccinations (Splenectomized Patients) | X | | | | | | | | | | | | |
| Physical Examination[5]/ Height[5] and Weight | X | | X | | X | | | X | X | X | X | X | X |
| Performance Status | X | | X | | X | | | X | X | X | X | X | X |

| **Timing** | **Pre-Treatment** | | **Month 1** | | | | **Months 2 and 3** | | | **Months 4, 5 and 6** | | | **Follow Up**[1] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | | **Baseline / D1** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day** | **-28 to -1** | **-26 to -1**[2] | **1** | **8**[3] | **15** | **22**[3] | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window** | | | | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| Vital signs (BP, HR, RR, T)[6] | X | | X | X | X | X | X | X | X | X | X | X | X |
| 12-lead ECG[7] | X | | X | X | | X | | | | | | X | X |
| DXA Scan[8] | X | | | | | | | | | | | X[9] | |
| Laboratory Evaluations[10] | | | | | | | | | | | | | |
| HBsAg, HCV Ab, HIV1 and 2 Ab | X | | | | | | | | | | | | |
| RBC antibody Screen | X | | | | | | | | | | | | |
| Hematology (CBC)[11] | X | X[12] | X | X | X | X | X | X | X | X | X | X | X |
| Haptoglobin[13] | | | X | | | X | | | X | | | X | X |
| EPO levels[14] | | | X | | | X | | | X | | | X | X |
| G6PD screen | X | | | | | | | | | | | | |
| Serum Chemistry[15] | X | | X | X | X | X | X | X | X | X | X | X | X |
| Iron Panel[16] | | | X | | | | | | X | | | X | |
| Carboxyhemoglobin (COHb) | | | X | | | X | X | X | X | X | X | X | |
| Coagulation Studies[17] | X | | X | | X | | | | X | | | X | X |
| Urinalysis[18] | X | | X | | X | | | | X | | | X | X |

| **Timing** | **Pre-Treatment** | | **Month 1** | | | | **Months 2 and 3** | | | **Months 4, 5 and 6** | | | **Follow Up**[1] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | | **Baseline / D1** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day** | **-28 to -1** | **-26 to -1**[2] | **1** | **8**[3] | **15** | **22**[3] | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window** | | | | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| Serum or Urine Pregnancy[19] | X | | X | | | | | | | | | | |
| Lipids[20] | | | X | | | | X | | X | | | X | X |
| Hormonal Testing[21] | X | X[22] | X | | | | | | X | | | X | X |
| Serum osteocalcin-N-mid and CTX[23] | | | X | | | | | | X | | | X | |
| 25-hydroxy Vitamin D2 and D3 | | | X | | | | | | X | | | X | |
| Randomization[24] | X | | | | | | | | | | | | |
| Study Drug Administration | | | X | X | X | X | X | X | X | X | X | X | |
| Dispense Study Drug[25] | | | X | X | X | X | X | X | X | X | X | | |
| PK blood sampling[26] | | | X | | X | X | X | X | X | X | X | X | |
| PD Assessments[26] | | | | | | | | | | | | | |
| 2,3-DPG/ATP | | | X | | X | X | X | X | X | X | X | X | |
| [redacted] | | | | | | | | | | | | | |
| Dispense/Collect Menstrual Cycle Diary[28] | | | X | | | | X | | X | X | X | X | X |
| Adverse Events[29] | | | Continuous | | | | | | | | | | X |

| **Timing** | **Pre-Treatment** | | **Month 1** | | | | **Months 2 and 3** | | | **Months 4, 5 and 6** | | | **Follow Up**[1] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | | **Baseline / D1** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day** | **-28 to -1** | **-26 to -1**[2] | **1** | **8**[3] | **15** | **22**[3] | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window** | | | | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| Transfusion Record[30] | X | | X | X | X | X | X | X | X | X | X | X | X |
| Concomitant Medications/Procedures | X | | X | X | X | X | X | X | X | X | X | X | X |
| Rollover to Extension Period[31] | | | | | | | | | | | | X | |

Abbreviations: Ab = antibody; ATP = adenosine triphosphate; BP = blood pressure; CBC= complete blood count; COHb = carboxyhemoglobin; CTX = C-terminal telopeptide; D = day; DPG = diphosphoglycerate; DXA = Dual-energy x-ray absorptiometry; ECG = electrocardiogram; EPO = erythropoietin; FSH = follicle stimulating hormone; G6PD = glucose-6-phosphate-dehydrogenase; HBsAg = hepatitis B surface antigen; HCV Ab = hepatitis C virus antibody; HDL-C = high-density lipoprotein-cholesterol; HIV = human immunodeficiency virus; HR = heart rate; PD = pharmacodynamic; PK = pharmacokinetic; PK deficiency = pyruvate kinase deficiency; PKR = pyruvate kinase isoform R; RR = resting rate; W = week.

*Whenever more than one assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (e.g., VS, ECG, blood draw). The timing of these assessments should allow the blood draw to occur at the exact nominal time. The order of procedures may be revised with prior discussion between Sponsor and site.*

[1] The Week 28 Follow-up Visit will only be conducted for patients who do not enter the Extension Period.

[2] To be performed at least 2 days after the first Screening Visit.

[3] In-clinic visits on Day 8 and Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis; in these instances PK/PD sampling would not be required and dispensing of study medication would not be performed. For the Day 8 visit performed by the patient's primary care physician, the primary care medical office will collect a blood sample for hematology using the blood sample collection and shipping supplies from the kit prepared by the designated central laboratory. The kit will be sent to the primary care physician's office. No other testing or procedures will be asked of the primary care physician on Day 8 [VS and serum chemistry will not be required]. The 12-lead ECG scheduled for Day 8 will instead be performed at the main study center on Day 15. For the Day 22 visit performed by the patient's primary care physician, the primary care medical office will collect a blood sample for hematology using the blood sample collection and shipping supplies from the kit prepared by the designated central laboratory. The kit will be sent to the primary care physician's office. No other testing or procedures will be asked of the primary care physician on Day 22. [VS, serum chemistry, coagulation, haptoglobin, EPO level, carboxyhemoglobin, and PK/PD samples will not be required.] The 12-lead electrocardiogram scheduled for Day 22 will instead be performed at the main study center on Day 43 (Week 6). For patients having their Day 8 and/or Day 22 visits performed by the primary care physician, the Principal Investigator will him or herself, or have a qualified research nurse or other designated site staff member, make telephone contact with the patient to inquire about any adverse events. These must be recorded as if the patient appeared in the main study center. The Principal Investigator will exercise prudent clinical judgment in determining any clinical course of action to take based on any adverse

events discovered. These telephone contacts on Day 8 and Day 22 must be explained to the patient in advance and scheduled in advance to maximize the likelihood of successfully making contact.

[4] Medical history, including all relevant prior medical history, current medical conditions, and hematology profile (CBCs) over prior 6 months, will be obtained at the Screening assessment.

[5] A complete physical examination (including neurological examination; genital and rectal examinations will be performed at the discretion of the Investigator) will be obtained at Screening, Baseline, Week 12, and the Follow up Visit (Week 28), or Week 24 for patients rolling over to the Extension Period. The neurological examination must include an assessment of general orientation and mental status including level of alertness (assess as normal or abnormal and specify any abnormality). Limited focused physical examinations will be performed at all other specified visits. Height to be collected at Screening only.

[6] Vital signs will include systolic and diastolic blood pressure, heart rate, respiratory rate, and temperature.

[7] 12-lead ECGs are to be conducted after 5 minutes of recumbency. Screening ECG will be performed at least 7 days prior to Day 1 dosing.

[8] If a DXA scan of the hip and spine with T and Z scores has been performed within 3 months preceding the first day of dosing, this may be used to meet this requirement.

[9] Week 24 DXA scan may be performed anytime between Weeks 24 and 28 and must be performed at the same imaging center on the same DXA machine as the original Screening DXA scan.

[10] Laboratory evaluations (hematology, serum chemistry, coagulation studies, and urinalysis) are to be collected in the morning. These should be collected following an overnight fast on Baseline Day 1 Week 6 (Day 43), Week 12 (Day 85), Week 24 (Day 169), and Follow-up Week 28 (Day 197), when the lipid samples are also included.

[11] Three Screening/Baseline samples will be collected for complete blood count (CBC). Samples will be collected in the AM on 3 different days; the samples collected on Baseline/Day 1 may comprise one of these; samples may be taken at the same time as Screening/Baseline hormone assessments (any 2 time points during Screening at least 2 days apart). CBC will include HCT, Hb, red blood cell (RBC) count, absolute reticulocyte count, percent reticulocyte count, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count with automated (or manual, as indicated) differential, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and platelet count. In the event that the designated central laboratory for the study is unable to provide a valid result for any specific component of the defined CBC for a specific patient, the site may be asked to have the test performed at their local laboratory. The result of the local CBC will be entered into the study database along with the local normal reference range.

[12] The second Screening hematology (CBC) should be drawn in the morning (does not have to be fasting), and may be drawn at the same time the patient returns for the second estradiol and free and total testosterone sample.

[13] Haptoglobin will be performed prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

[14] Erythropoietin (EPO) levels will be performed prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

[15] Alkaline phosphatase, sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide (CO2) or bicarbonate, albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and indirect bilirubin, (and estimated creatinine clearance or glomerular filtration rate for screening only, as appropriate).

[16] Iron, total iron-binding capacity (TIBC), transferrin saturation, and ferritin will be performed prior to dosing on Day 1, at the end of Week 12 and at the end of Week 24.

[17] Fibrinogen, activated partial thromboplastin time (aPTT), and international normalized ratio (INR) will be performed at Screening, prior to dosing on Day 1, at the end of Week 2, the end of Week 12, the end of Week 24, and the end of Week 28.

[18] Color, appearance, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, and occult blood. Microscopic inspection of sediment should only be performed for cause or to investigate an abnormal dipstick finding per the Investigator's discretion. Urinalysis will be performed at Screening, prior to dosing on Day 1, at the end of Week 2, the end of Week 12, the end of Week 24, and the end of Week 28.

[19] Must be repeated at any point throughout the study period if pregnancy is clinically suspected.

[20] Total cholesterol, HDL-C, and triglyceride samples will be collected in the morning following an overnight fast.

[21] Three Screening/Baseline samples will be collected for estradiol and testosterone (total and free). Samples will be collected in the AM on 3 different days; the samples collected on Baseline/Day 1 may comprise one of these; the Screening samples may be collected at any 2 time points during Screening at least 2 days apart. Serum estrone, estradiol, and free and total testosterone will then follow the schedule indicated on Day 1 and Weeks 12, 24, and 28. FSH will only be performed at Screening for female patients only for confirmation of post-menopausal status.

[22] The second Screening hormone testing will consist of estradiol and testosterone (free and total) only. Samples should be drawn in the AM (does not need to be fasting), and may be drawn at the same time the patient returns for the second CBC sample.

[23] Serum osteocalcin-N-mid and CTX will be drawn in the AM each time, approximately between 8-10 AM, and after an overnight fast of 10-12 hours.

[24] Randomization will be performed following PKR genotyping and prior to and as close as feasible to dosing on Day 1.

[25] Study drug will be dispensed on a 28-day schedule, or on an alternate schedule (< 28 days) as needed to accommodate patient visit schedule and dose modifications.

[26] For the first 10 patients treated, extensive PK/PD sampling will be conducted on Days 1 and 15 (see Appendix 15.1, Table 7 for details), followed by limited PK/PD sampling from Week 3 to Week 24 (see Appendix 15.1, Table 8 for details). Limited PK/PD sampling will be conducted on the remainder of patients treated (see Appendix 15.1, Table 8). See Section 10.6, Section 10.7, and Section 10.9 for details on blood sampling for PK and PD assessments, respectively, and guidelines on sample processing and storage.

[28] Menstruating female patients will record their menstrual cycles (start, stop, characteristics) monthly. Paper-based menstrual cycle diaries will be dispensed at study visits approximately every month. The previous month's diary will be collected at these visits as well.

[29] All randomized patients will be evaluated for AEs from the time they sign informed consent until they complete all scheduled study follow-up visits or withdraw consent, whichever occurs first.

[30] All transfusions must be recorded in the eCRF

[31] Patient must have completed 24 weeks of treatment and tolerated AG-348 (may have had dose modifications). Investigator and Medical Monitor or Responsible Medical Officer must agree with the patient continuing on treatment and patient must sign a separate ICF for the Extension Period.

**Table 6: Schedule of Assessments: Extension Period**

| Visit | Month 9 | Month 12 | Month 15 | Month 18 | Month 21 | Month 24 | Month 27 | Month 30 | Follow-up |
|---|---|---|---|---|---|---|---|---|---|
| **Approximate Study Day** | **259** | **365** | **455** | **545** | **635** | **724** | **814** | **904** | **934** |
| **Visit Window** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** |
| Physical Examination/Weight[1] | X | X | X | X | X | X | X | X | X |
| Performance Status | X | X | X | X | X | X | X | X | X |
| Vital signs (BP, HR, RR, T)[2] | X | X | X | X | X | X | X | X | X |
| 12-lead ECG[3] | | X | | X | | X | | X | X |
| DXA Scan | | | | X | | | | X | |
| Laboratory Evaluations[4] | | | | | | | | | |
| Hematology (CBC)[5] | X | X | X | X | X | X | X | X | X |
| Haptoglobin | | X | | X | | X | | X | X |
| EPO levels[6] | | X | | X | | X | | X | X |
| Serum Chemistry[7] | X | X | X | X | X | X | X | X | X |
| Iron Panel[8] | | X | | X | | X | | X | |
| Carboxyhemoglobin (COHb) | | X | | X | | X | | X | |
| Coagulation Studies[9] | X | X | X | X | X | X | X | X | X |
| Urinalysis[10] | X | X | X | X | X | X | X | X | X |
| Serum or Urine Pregnancy[11] | | | | | | | | | |
| Lipids[12] | X | X | X | X | X | X | X | X | X |
| Hormonal Testing[13] | X | X | X | X | X | X | X | X | X |
| Serum osteocalcin-N-mid and CTX[14] | | | | X | | | | X | |
| Study Drug Administration | X | X | X | X | X | X | X | X | |

| Visit | Month 9 | Month 12 | Month 15 | Month 18 | Month 21 | Month 24 | Month 27 | Month 30 | Follow-up |
|---|---|---|---|---|---|---|---|---|---|
| **Approximate Study Day** | **259** | **365** | **455** | **545** | **635** | **724** | **814** | **904** | **934** |
| **Visit Window** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** | **± 2 W** |
| Dispense Study Drug[15] | X | X | X | X | X | X | X | | |
| PK blood sampling[16] | X | X | X | X | X | X | X | X | |
| PD Assessments[17] (2,3-DPG/ATP, [redacted]) | X | X | X | X | X | X | X | X | |
| Dispense/Collect Menstrual Cycle Diary[18] | X | X | X | X | X | X | X | X | X |
| Adverse Events[19] | Continuous | | | | | | | | X |
| Transfusion Record[20] | X | X | X | X | X | X | X | X | X |
| Concomitant Medications/Procedures | X | X | X | X | X | X | X | X | X |

Abbreviations: ATP = adenosine triphosphate; BP = blood pressure; CBC= complete blood count; COHb = carboxyhemoglobin; CTX = C-terminal telopeptide; DPG = diphosphoglycerate; DXA = Dual-energy x-ray absorptiometry; ECG = electrocardiogram; EPO = erythropoietin; HDL-C = high-density lipoprotein-cholesterol; HIV = human immunodeficiency virus; HR = heart rate; PD = pharmacodynamic; PK = pharmacokinetic; PK deficiency = pyruvate kinase deficiency; PKR = pyruvate kinase isoform R; RR = resting rate; W = week.

*Whenever more than one assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (e.g., VS, ECG, blood draw). The timing of these assessments should allow the blood draw to occur at the exact nominal time. The order of procedures may be revised with prior discussion between Sponsor and site.*

[1] A focused physical examination (including neurological examination; genital and rectal examinations will be performed at the discretion of the Investigator) will be obtained every 3 months; a complete physical examination will be performed at the Month 30 visit. The neurological examination must include an assessment of general orientation and mental status including level of alertness (assess as normal or abnormal and specify any abnormality).

[2] Vital signs will include systolic and diastolic blood pressure, heart rate, respiratory rate, and temperature

[3] 12-lead ECGs are to be conducted after 5 minutes of recumbency.

[4] Laboratory evaluations (hematology, serum chemistry, coagulation studies, and urinalysis) are to be collected in the morning. These should be collected following an overnight fast.

[5] CBC will include HCT, Hb, red blood cell (RBC) count, absolute reticulocyte count, percent reticulocyte count, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count with automated (or manual, as indicated) differential, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and platelet count. In the event that the designated central laboratory for the study is unable to provide a valid result for any specific component of the defined CBC for a specific patient, the site may be asked to have the test performed at their local laboratory. The result of the local CBC will be entered into the study database along with the local normal reference range.

[6] Erythropoietin (EPO) levels will be performed prior to dosing at Month 12, Month 18, Month 24, and Month 30.

[7] Alkaline phosphatase, sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide (CO2) or bicarbonate, albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and indirect bilirubin, (and estimated creatinine clearance or glomerular filtration rate, as appropriate).

[8] Iron, total iron-binding capacity (TIBC), transferrin saturation, and ferritin will be performed prior to dosing at Month 12, Month 18, Month 24, and Month 30.

[9] Fibrinogen, activated partial thromboplastin time (aPTT), and international normalized ratio (INR) will be performed at each study visit.

[10] Color, appearance, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, and occult blood. Microscopic inspection of sediment should only be performed for cause or to investigate an abnormal dipstick finding per the Investigator's discretion. Urinalysis will be performed prior to dosing at each study visit.

[11] Must be obtained at any point throughout the study period if pregnancy is clinically suspected. Effective contraception must be continued throughout the Extension Period in all patients and any pregnancies must be reported (see Section 11.3).

[12] Total cholesterol, HDL-C, and triglyceride samples will be collected in the morning following an overnight fast.

[13] Serum estrone, estradiol, and free and total testosterone.

[14] Serum osteocalcin-N-mid and CTX will be drawn in the AM each time, approximately between 8-10 AM, and after an overnight fast of 10-12 hours.

[15] Study drug will be dispensed on a 3-month schedule, or on an alternate schedule (< 3 months) as needed to accommodate patient visit schedule and dose modifications.

[16] Predose; PK sampling will only include AG-348 and AGI-8702 concentrations.

[17] Predose.

[18] Menstruating female patients will record their menstrual cycles (start, stop, characteristics) monthly. Paper-based menstrual cycle diaries will be dispensed and collected at each study visit.

[19] All randomized patients will be evaluated for AEs from the time they sign informed consent until they complete all scheduled study follow-up visits or withdraw consent, whichever occurs first.

[20] All transfusions must be recorded in the eCRF.

**Table 7: Schedule of Assessments: Extensive PK/PD Sampling during the Core Period**

| **Sample Timing/Interval** | **Month 1** | | | | | | | | **Months 2 and 3** | | | **Months 4, 5 and 6** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Baseline / D1 W2 / D15** | | | | | | | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** |
| **Study Day** | **1/15** | | | | | | | **22** | **43** | **64** | **85** | **113** | **141** | **169** |
| **Visit Window** | **± 2 D (D15)** | | | | | | | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| **Timing** | Pre-dose[1] | 30 min[2] | 1 hr[2] | 2 hr[2] | 4 hr[3] | 8 hr[3] | 12 hr[3] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] |
| PK blood sample | X | X | X | X | X | X | X[4] | X | X | X | X | X | X | X |
| 2,3 DPG/ATP | X | X | X | X | X | X | X[4] | X | X | X | X | X | X | X |
| [redacted] | | | | | | | | | | | | | | |

Abbreviations: ATP = adenosine triphosphate; D = day; DPG = diphosphoglycerate; PD = pharmacodynamic; PK = pharmacokinetic; [redacted] W = week.

[1] The acceptable time window will be within 60 minutes prior to study treatment dose administration for the pre-dose PK/PD sample.

[2] The acceptable time window will be within ± 5 minutes of the scheduled collection time for the 30 minute, 1 and 2 hour PK/PD samples.

[3] The acceptable time window will be within ± 30 minutes of the scheduled collection time for the 4, 8, and 12 hour PK/PD samples.

[4] To be collected on Day 1 only.

[redacted]

[6] If the 12 hour time point cannot be collected at site on Day 1, an 8 hour time point may be collected instead.

[redacted]

**Table 8: Schedule of Assessments: Limited PK/PD Sampling during the Core Period**

| Sample Timing/Interval | Month 1 | | | Months 2 and 3 | | | Months 4, 5 and 6 | | |
|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Baseline / D1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** |
| **Study Day** | **1** | **15** | **22** | **43** | **64** | **85** | **113** | **141** | **169** |
| **Visit Window** | **-** | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| **Timing** | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] |
| PK blood sample | X | X | X | X | X | X | X | X | X |
| 2,3 DPG/ATP | X | X | X | X | X | X | X | X | X |
| [redacted] | | | | | | | | | |

Abbreviations: ATP = adenosine triphosphate; D = day; DPG = diphosphoglycerate; PD = pharmacodynamic; PK = pharmacokinetic; [redacted] W = week.

[1] The predose blood sample for plasma PK/PD analysis should be collected within 60 minutes prior to study treatment dose administration.

[redacted]

## 15.2. Eastern Cooperative Oncology Group Performance Status Scoring

| Grade | Symptomatology |
|---|---|
| 0 | Fully active, able to carry on all pre-disease performance without restriction |
| 1 | Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work |
| 2 | Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours |
| 3 | Capable of only limited self-care, confined to bed or chair more than 50% of waking hours |
| 4 | Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair |
| 5 | Dead |

Source: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and Response Criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-655.

## 15.3. National Cancer Institute Common Terminology Criteria for Adverse Events

The NCI CTCAE, Version 4.03, can be accessed using the following link:

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

## 15.4. Potential Drug Interactions

Strong inhibitors of CYP3A4 (listed in Table 9) are not permitted for use with AG-348. Based on modeling of AG-348 metabolism and tolerability data from the Phase 1 MAD study, it is thought that moderate inhibitors of CYP3A4 do not pose a risk to patients dosed with AG-348.

In-vivo data from the Phase 1 MAD show AG-348 induces CYP3A4 and induces its own metabolism, presumably via CYP3A4. The use of strong CYP3A4 inducers in combination with AG-348 is expected to reduce AG-348's efficacy. Therefore, administration of strong CYP3A4 inducers (listed in Table 10) with AG-348 is not permitted. Strong inhibitors of drug transport (listed in Table 11) are not permitted for use with AG-348.

Digoxin is not permitted for use with AG-348.

Induction of CYP3A4 by AG-348 is expected to reduce the efficacy of certain sensitive co-medications. Co-administration of AG-348 with the drugs in Table 12 should be done with caution, as their efficacy may be reduced.

Of note, in accordance with Inclusion Criteria 14, women in the trial utilizing oral contraception must utilize barrier methods while taking AG-348.

The expected patient co-medications deferoxamine, deferasirox, deferiprone, and oral penicillin are not expected to interact with AG-348.

**Table 9: Strong and Moderate CYP3A4 Inhibitors**

| Strong CYP3A4 Inhibitors: Contraindicated | Moderate CYP3A4 Inhibitors: No Action |
|---|---|
| Indinavir | Aprepitant |
| Nelfinavir | Erythromycin[1] |
| Ritonavir | Fluconazole |
| Clarithromycin | Verapamil[1] |
| Itraconazole | Diltiazem[1] |
| Ketoconazole | |
| Nefazodone | |
| Saquinavir | |
| Suboxone | |
| Telithromycin | |
| Grapefruit juice[2] | |

Strong Inhibitor; > 5 fold increase in AUC

Moderate Inhibitor; > 2 fold, < 5 fold increase in AUC

[1] Erythromycin, verapamil and diltiazem are contraindicated because they are strong P-gp inhibitors (see Table 11)

[2] Although classified as a moderate CYP3A4 inhibitor, grapefruit and grapefruit juice are prohibited

**Table 10: Strong CYP3A4 Inducers**

| **Strong CYP3A4 Inducers: Contraindicated** | |
|---|---|
| Efavirenz | Phenytoin |
| Nevirapine | Pioglitazone |
| Carbamazepine | Rifabutin |
| Glucocorticoids[1] | Rifampin |
| Modafinil | St. John's Wort |
| Oxcarbazepine | Troglitazone |
| Phenobarbital | |

[1] Short-term (≤ 14 days at a time, and ≤ 28 days total during the 24 week treatment period) use of topical, inhaled, intra-nasal, and systemic glucocorticoids is permitted for acute medical indications. Every effort should be made to minimize total duration of glucocorticoid therapy and utilize alternative treatments. Patients must be off glucocorticoids for at least 28 days prior to Day 1 of AG-348 dosing as per exclusion criterion #11 (Section 8.3). For patients who require chronic inhaled glucocorticoid therapy, Investigators should confer with the Medical Monitor for additional guidance.

**Table 11: Strong P-glycoprotein Inhibitors**

| **Strong P-gp Inhibitors: Contraindicated** | |
|---|---|
| Amiodarone | Felodipine |
| Azithromycin | Itraconazole |
| Captopril | Ketoconazole |
| Carvedilol | Lopinavir |
| Clarithromycin | Ritonavir |
| Conivaptan | Quercetin |
| Cyclosporine | Quinidine |
| Diltiazem | Ranolazine |
| Dronedarone | Ticagrelor |
| Erythromycin | Verapamil |

**Table 12: Sensitive CYP3A4 Substrates**

| **Sensitive CYP3A4 Substrates: Substitute or Use with Caution** | | | | |
|---|---|---|---|---|
| | **Antihistamines:** | **Miscellaneous:** | | |
| | Chlorpheniramine | Alfentanil | Finasteride | Salmeterol |
| | | Aprepitant | Gleevec | Sildenafil |
| **Benzodiazepines:** | **Calcium Channel Blockers:** | Aripiprazole | Haloperidol | Sirolimus |
| Alprazolam | Amlodipine | Boceprevir | Irinotecan | Sorafenib |
| Diazepam→3OH | Lercanidipine | Buspirone | LAAM | Sunitinib |
| Midazolam | Nifedipine | Cafergot | Lidocaine | Tamoxifen |
| Triazolam | Nisoldipine | Caffeine→TMU | Methadone | Taxol |
| | Nitrendipine | Cilostazol | Nateglinide | Telaprevir |
| **Immune Modulators:** | | Cocaine | Nevirapine | Terfenadine |
| Tacrolimus (FK506) | **HMG CoA Reductase Inhibitors**: | Codeine-N-demethylation | Ondansetron | Torisel |
| | Atorvastatin | Dapsone | Pimozide | Trazodone |
| **Steroid 6beta-OH:** | Cerivastatin | Dextromethorphan | Propranolol | Vemurafenib |
| Estradiol | Lovastatin | Docetaxel | Quetiapine | Vincristine |
| Hydrocortisone (and other glucocorticoids) | Simvastatin | Domperidone | Quinine | Zaleplon |
| Progesterone | | Eplerenone | Risperidone | Ziprasidone |
| Testosterone | | Fentanyl | Romidepsin | Zolpidem |

## 15.5. Declaration of Helsinki

**WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI:**

Recommendations Guiding Medical Doctors in Biomedical Research Involving Human Subjects

Adopted by the 18th World Medical Association (WMA) General Assembly, Helsinki, Finland, June 1964 and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975, 35th WMA General Assembly, Venice, Italy, October 1983, and the 41st WMA General Assembly, Hong Kong, September 1989, the 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996; 52nd WMA General Assembly, Edinburgh, Scotland, October 2000; 53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added); 55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added); and 59th WMA General Assembly, Seoul, October 2008.

**A. INTRODUCTION**

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.
   The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of subjects, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my subject will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the subject's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
8. In medical practice and in medical research, most interventions involve risks and burdens.

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.
10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

**B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH**

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.
12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, Sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.
15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the Sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any SAEs. No change to the protocol may be made without consideration and approval by the committee.
16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on subjects or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects

must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.
25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.
27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.
28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious subjects, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.
30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

## C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the subjects who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:
    - The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
    - Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the subjects who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.
33. At the conclusion of the study, subjects entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.
34. The physician must fully inform the subject which aspects of the care are related to the research. The refusal of a subject to participate in a study or the subject's decision to withdraw from the study must never interfere with the subject-physician relationship.
35. In the treatment of a subject, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the subject or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

agios

# Clinical Study Protocol AG348-C-003
# EudraCT No. 2015-000484-13
# A Phase 2, Open Label, Randomized, Dose Ranging, Safety, Efficacy, Pharmacokinetic and Pharmacodynamic Study of AG-348 in Adult Patients with Pyruvate Kinase Deficiency

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|---|---|
| Study Sponsor: | Agios Pharmaceuticals, Inc.<br>88 Sidney Street<br>Cambridge, MA 02139-4169<br>Phone: 617-649-8600<br>Fax: 617-649-8618 |
| Responsible Medical Officer: | [redacted], MD, PhD<br>[redacted]<br>Agios Pharmaceuticals, Inc.<br>Phone: [redacted]<br>Email: [redacted] |
| Study Medical Monitor | [redacted], MD<br>[redacted]<br>On behalf of Agios Pharmaceuticals, Inc.<br>Mobile Phone: [redacted]<br>Office Phone: [redacted]<br>Email: [redacted] |
| Document Version (Date):<br>Revised | Version 1.0 (05 January 2015)<br>Amendment 1, Protocol Version 2.0 (02 February 2015) Final<br>Amendment 2, Protocol Version 3.0 (05 Aug 2015) Final |

This study will be conducted according to the protocol and in compliance with Good Clinical Practice, the ethical principles stated in the Declaration of Helsinki, and other applicable regulatory requirements.

**CONFIDENTIALITY NOTE:**

The information contained in this document is confidential and proprietary to Agios Pharmaceuticals, Inc. Any distribution, copying, or disclosure is strictly prohibited unless such disclosure is required by federal regulations or state law. Persons to whom the information is disclosed must know that it is confidential and that it may not be further disclosed by them.

# INVESTIGATOR'S AGREEMENT

I understand that all documentation provided to me by Agios or its designated representative(s) concerning this study that has not been published previously will be kept in strict confidence. This documentation includes the study protocol, Investigator's Brochure, case report forms, and other scientific data.

This study will not commence without the prior written approval of a properly constituted Institutional Review Board (IRB). No changes will be made to the study protocol without the prior written approval of Agios and the IRB, except where necessary to eliminate an immediate hazard to the patient.

I have read, understood, and agree to conduct this study as outlined in the protocol and in accordance with the guidelines and all applicable government regulations.

| Investigator Name (printed) | Investigator Signature | Date |
|---|---|---|

Investigational site or name of institution and location (printed)

# 2. SYNOPSIS

**Name of Sponsor/Company:**
Agios Pharmaceuticals, Inc.

**Name of Investigational Product:**
AG-348

**Name of Active Ingredient:**
AG-348 sulfate hydrate

**Title of Study:**
A Phase 2, Open Label, Randomized, Dose Ranging, Safety, Efficacy, Pharmacokinetic and Pharmacodynamic Study of AG-348 in Adult Patients with Pyruvate Kinase Deficiency

**Study Center(s):**
This study will be conducted at multiple study centers.

**Phase of Development:** 2

***Objectives:***

**Primary:**

- Evaluate the safety and tolerability of up to 24 weeks of AG-348 administration in patients with pyruvate kinase deficiency (PK deficiency).

**Secondary:**

- Evaluate the pharmacokinetics (PK) of AG-348 and the metabolite AGI-8702.
- Evaluate the pharmacodynamic (PD) response of adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG) after administration of AG-348.
- Evaluate indicators of clinical activity of AG-348 in patients with PK deficiency, including changes in hemoglobin (Hb), hematocrit (HCT), reticulocyte count, haptoglobin (Hp), carboxyhemoglobin (COHb), lactate dehydrogenase (LDH), total and indirect bilirubin, erythropoietin (EPO), ferritin, and transferrin saturation (serum iron/iron binding capacity).

[redacted]

**Methodology:**
Study AG348-C-003 is a Phase 2, open label, two arm, multicenter, randomized, dose-ranging study during which adult patients with PK deficiency will receive multiple doses of AG-348 for up to 24 weeks. Patients with PK deficiency confirmed by red blood cell PK enzymatic assay performed at Screening will be eligible to participate in this study. At Week 25, patients who safely tolerate AG-348 and demonstrate clinical activity of AG-348 may be eligible to immediately roll over to a safety extension for continued treatment. Patients who finish treatment at the end of 24 weeks or sooner will undergo follow-up assessment 4 weeks after the last dose of study drug. Patients with toxicity suspected to be related to study drug will continue follow-up until the adverse event (AE) resolves, is declared chronic by the Investigator, or the patient is lost to follow-up.

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Initially, up to 25 patients will be randomized on an open-label 1:1 basis to each of two twice-daily (BID) doses of AG-348 (up to 50 patients total; see Figure 1, Study Schema). The dose of Arm 1 is 300 mg of AG-348 administered orally every 12 hours (q12h, BID). The dose of Arm 2 is 50 mg of AG-348 administered orally q12h (BID). Randomization will be stratified by *PKR* mutation in order to maintain balance as much as possible across the dose arms for the specific mutations expected to be most frequently enrolled. The *PKR* mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H) and all other mutations ("other"). Mutation status is defined by the presence of at least one of the indicated mutations; patients with more than one stratified mutation will be assigned based on Sponsor's discretion.

The doses for each arm have been selected from the forerunner AG348-C-001 single ascending dose (SAD) and AG348-C-002 multiple ascending dose (MAD) studies in healthy adult volunteers to represent the range of doses/exposures that were safely tolerated and resulted in maximal or near-maximal PD effects on 2,3-DPG and ATP.

Because PK deficiency is a rare disease with a limited eligible patient population and because the underlying pathophysiology and clinical phenotype of affected patients is heterogenous due to the wide variety of mutations in PKR that cause the disease, it is important to focus closely on dose findings in this first-in-patient study. Therefore, in addition to initiating this study with 2 different doses of AG-348 administered q12h, a Data Review Team (DRT) will be established to review study data on a regular basis and adapt the study design, dose and schedule of AG-348.

The DRT will monitor safety on an on-going basis and meet at regular intervals (approximately every 6 weeks), or *ad* hoc, as necessary, to review AEs, vital signs (VS), clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and electrocardiograms (ECGs) on enrolled patients. The DRT will also review available PK/PD data and indicators of clinical activity (e.g., changes from baseline in Hb). The DRT will be comprised of the study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer.

Approximately every 6 weeks beginning six weeks after the first patient is dosed or *ad hoc* as necessary, the DRT will review cumulative safety data, available PK/PD data, and clinical activity data. Based on the DRT's recurring 6 week reviews, the DRT may exercise one or more of the following options:

- Continue treatment and enrollment in existing arms without change.
- Add 1 new dose arm (Arm 3) to enroll up to 25 patients at a dose to be determined; the dose for Arm 3 may be lower or higher than Arm 1 and Arm 2 doses, but will not exceed 360 mg q12h; and the dose regimen may be less frequent than q12h.
- Terminate or suspend enrollment to allow further review of clinical data in Arm 1 and/or Arm 2 (and/or potential Arm 3). Enrollment in an arm could be terminated or suspended to allow further review, for example, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity.
- Re-assign patients' doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the patients in the terminated arm will remain in their original arm, i.e., they will not count towards the enrollment quota of the arm whose dose and

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schedule is being adopted.

- Implement specific genotype restrictions to enrollment in one or more arms to ensure representation of patients with genotypes of greatest clinical relevance.

The DRT will perform these evaluations on a recurring 6-week basis. The data that the DRT will review to make these decisions is expected to include, but are not necessarily limited to, the following:

- *Safety Observations:* all AEs; VS, clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and ECG;
- *PK and PD Observations:* including changes in 2,3-DPG and ATP;
- *Indicators of Clinical Activity:* including changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, EPO, total and indirect bilirubin, ferritin, and transferrin saturation.

If a third dose arm is implemented, the dose of AG-348 selected will not exceed 360 mg BID, as this was the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy volunteers. The PK/PD sampling schedule in a potential third dosing arm will be determined by Sponsor's discretion and may follow either the extensive or limited PK/PD sampling schedules as specified.

The DRT will monitor the data in an ongoing manner as described and may make a decision to terminate enrollment in an arm if unacceptable AEs are observed, PD activity is limited, and/or markers of clinical activity indicate no effect. Unacceptable AEs are defined as ≥ Grade 3 AEs (using National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAEv4.03]) that are assessed as at least possibly related to AG-348, with the exception of Grade 3 headache, hot flash/flushing, nausea, vomiting, and/or diarrhea that is transient in duration (< 24 hours) or able to be medically managed to ≤ Grade 2 within 24 hours. The DRT may also exercise discretion to terminate enrollment in an arm if it observes a frequency of Grade 2 AEs that would make it difficult to support long-term dosing.

Due to the potential for AG-348-mediated aromatase inhibition, dual-energy x-ray absorptiometry (DXA) scan (hip and spine) will be performed at Screening (if patient has not had prior DXA scan within 3 months of Day 1) to obtain T and Z scores. These data are intended to serve as a baseline measure of bone mineral density for all enrolling patients, and are deemed of particular importance for those who may enter the longer term safety extension after completing the current study. All patients will have a second DXA scan in the interval between Weeks 24 and 28. The safety extension will address additional follow-up DXA scanning.

As the number of enrolling arms changes in the study (for example, from 2 to 3), the randomization scheme will adjust to enable balanced randomization into each actively accruing arm. Randomization and stratification will cease in the event that only a single arm is left enrolling.

Depending on possible early termination of 1 or both of the initial 2 arms, or the addition of a third arm, the study could enroll up to a maximum of 75 patients.

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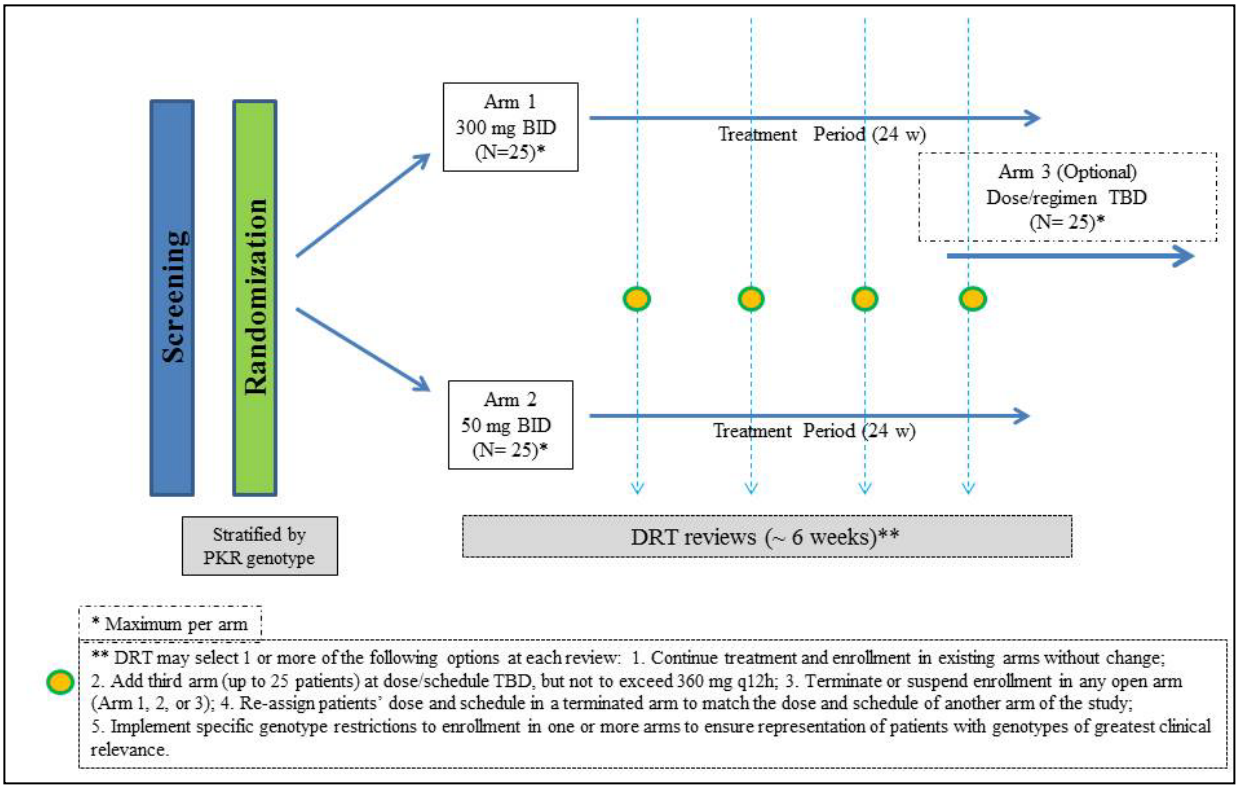
**Figure 1: Study Schema**




Abbreviations: BID (q12h) = twice-daily (every 12 hours); DRT = data review team; PKR = pyruvate kinase red blood cell isoform; TBD= to be determined; w = week(s).

***Visit Schedule***

Screening assessments will occur within 28 days prior to the first dose of study treatment. During the Treatment period, patients will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12), and monthly through Week 24 (Weeks 16, 20, and 24). Patients who safely tolerate AG-348 and demonstrate evidence of clinical activity of AG-348 through Week 24 may be eligible to immediately enter a safety extension for continued treatment. For patients who finish treatment at the end of 24 weeks or sooner, or who elect not to enter the safety extension, study discharge will occur 4 weeks (Week 28 or earlier) following the last dose of study treatment at the final follow-up assessment.

**Dose Modifications for Safety and/or Increase in Hb Level**

The Investigator will monitor all patients for safety and tolerability. Modification of an individual patient's dose of AG-348 will be based on AEs and/or observed changes in Hb level as described in Section 9.7.1 and Section 9.7.2.

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**Number of patients (planned):** Up to approximately 75 patients.

**Diagnosis and main criteria for inclusion:**

**Inclusion criteria:**

For entry into the study, patients must meet all of the following criteria during the Screening or other specified period:

1. Signed written informed consent obtained prior to performing any study procedure, including screening procedures.
2. Male or female, aged 18 years and older.
3. Known medical history of PK deficiency.
4. All patients must have documented clinical laboratory confirmation of PK deficiency by red blood cell pyruvate kinase enzymatic assay performed at Screening by a designated central laboratory. Patients with prior documentation of PK deficiency by red blood cell (RBC) enzymatic assay will have a reconfirmation of this result during Screening as a condition of enrollment.
    a. In the event that a patient's screening pyruvate kinase enzymatic assay is negative (i.e., shows normal pyruvate kinase activity), the patient will be eligible for enrollment if the genotyping shows a mutant genotype that has been previously documented in the literature to be associated with pyruvate kinase deficiency. If the genotyping shows a previously undescribed mutation in the PKR gene, then the eligibility for enrollment will be determined on a case-by-case basis by the Coordinating Investigator and Medical Monitor in discussion with the Investigator. If no mutation is defined, then the patient will not be eligible.
5. All patients must have genotypic characterization of the mutant PKR gene performed by a designated central laboratory at Screening, unless genotype is available from the patient's participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480).
6. All patients must have genotypic characterization of the UGT1A1 gene performed by a designated central laboratory to document whether they may have underlying Gilbert's Disease. Patients with Gilbert's Disease are eligible to enroll.
7. Males must have Hb $\leq$ 12.0 g/dL; females must have Hb $\leq$ 11.0 g/dL.
8. All patients must be considered transfusion independent as defined by: no greater than 3 units of RBCs transfused in the 12-month period up to the first day of study dosing and no transfusions within 4 months of first day of study dosing. Patients who have received more transfusion support than described above will evaluated for eligibility on a case-by-case basis by the

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Medical Monitor.

9. Eligible patients may still have their spleens in place, or may have undergone prior splenectomy. For splenectomized patients:
    a. Must have undergone their procedure at least 6 months prior to Screening.
    b. Must be current in their vaccinations for Pneumococcal Conjugate (PCV13), Pneumococcal Polysaccharide (PPSV23), Quadrivalent Meningococcal vaccine, and *Haemophilus influenzae* type b (Hib) as recommended by Centers for Disease Control and Prevention's (CDC) Advisory Committee on Immunization Practices (ACIP) or immunization advisory groups in Canada and the European Union (for patients enrolled in Canada and the EU). [http://www.cdc.gov/vaccines/schedules/downloads/adult/adult-schedule.pdf] [Any missing vaccinations may be administered during the screening period.]
10. Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 2. (Appendix 15.2)
11. Patients must be taking at least 1 mg of folic acid daily for at least 21 days prior to first dose and continue daily during study participation.
12. Adequate organ function, defined as:
    a. Serum aspartate transaminase (AST) and alanine aminotransferase (ALT) ≤ 1.5 × upper limit of normal (ULN) (unless the increased AST is assessed by the Investigator as due to hemolysis).
    b. Normal or elevated levels of serum bilirubin. In patients with serum bilirubin > ULN, the elevation must be attributed to hemolysis with or without Gilbert's syndrome and must not be choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease.
    c. Serum creatinine ≤ 1.25 × ULN. If serum creatinine > 1.25 × ULN, then 24-hour measured or calculated (Cockcroft-Gault) glomerular filtration rate (GFR) ≥ 60 mL/min.
    d. Absolute neutrophil count (ANC) > $1.0 \times 10^9$/L.
    e. Platelet count ≥ $100 \times 10^9$/L.
    f. Activated partial thromboplastin time (aPTT) and international normalized ratio (INR) ≤ 1.25 × ULN, unless the patient is receiving therapeutic anticoagulants.
13. Women of childbearing potential (WOCBP) must agree to abstain from sexual intercourse or to use an acceptable/effective method of contraception (i.e., condom plus spermicide, condom plus oral contraceptive, condom plus intrauterine device [IUD], condom plus diaphragm with spermicide) from as soon as feasible during the Screening period until 30 days following the last dose of AG-348. Abstinence is an acceptable method only when this is in line with the normal life style of the patient, meaning that the patient plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence, e.g., calendar, sympathothermal and post-ovulation methods, and

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withdrawal are not acceptable methods of contraception.

a. WOCBP include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, and/or bilateral oophorectomy) or is not post-menopausal. Post-menopausal is defined as:
   i. Amenorrhea ≥ 12 consecutive months without another cause, and a documented serum follicle stimulating hormone (FSH) level > 35 mIU/mL;
   ii. Amenorrhea ≥ 12 consecutive months in women ≥ 62 years old (FSH testing is not required).

14. WOCBP must have a negative serum or urine pregnancy test within 72 hours before start of AG-348 dosing.
15. Women must not be breastfeeding.
16. Male patients, with the exception of those who have undergone vasectomy at least 6 months prior to Screening, must agree to abstain from sexual intercourse or, if sexually active, to use a condom with spermicide as contraception (regardless of their female partner's childbearing potential or their partner's use of their own contraception) from Day 1 of dosing until 30 days following the last dose of AG-348. Abstinence is an acceptable method only when this is in line with the normal life style of the patient, meaning that the patient plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence, e.g., selective timing of intercourse based on partner's calendar, sympathothermal and post-ovulation methods, and withdrawal are not acceptable methods of contraception.

**Exclusion criteria:**

Patients who meet any of the following criteria at Screening or prior to dosing on Day 1 will not be enrolled in the study:

1. Hemoglobin level > 12.0 g/dL if male; Hb > 11.0 g/dL if female.
2. Additional diagnosis of any other congenital or acquired blood disorder, including glucose-6-phosphate-dehydrogenase (G6PD) deficiency, or any other hemolytic anemia process except for mild allo-immunization as a consequence of transfusion therapy.
3. Iron overload (hemosiderosis or concurrent hemochromatosis) sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac, hepatic, or pancreatic insufficiency.
4. Prior bone marrow or stem cell transplant.
5. Clinically symptomatic cholelithiasis or cholecystitis. (Prior cholecystectomy is not exclusionary. Patients with symptomatic cholelithiasis or cholecystitis may be rescreened once

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the disorder has been treated and clinical symptoms have resolved.)

6. Currently enrolled in another therapeutic clinical trial involving on-going therapy with any investigational or marketed product or placebo. Concurrent participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480) is permitted.
7. Exposure to any investigational drug, device, or procedure within 28 days prior to Screening.
8. Concurrent medical condition that could compromise participation in the study such as:
    a. Poorly controlled hypertension (defined as systolic blood pressure (BP) > 150 mm Hg or diastolic BP > 90 mm Hg) refractory to medical management.
    b. History of recent (within < 6 months from Screening date) congestive heart failure; myocardial infarction or unstable angina pectoris; or hemorrhagic, embolic, or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.
    c. Currently active infection requiring the use of parenteral anti-microbial agents or that is greater than Grade 3 (CTCAEv4.03) within 6 months of first dose.
    d. A pattern or frequency of post-splenectomy sepsis that in the assessment of the Investigator could reasonably be expected to interfere with the ability of the patient to complete the 24 week study participation.
    e. Positive test for hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV) antibody with signs of active Hepatitis B or C virus infection.
    f. Positive test for human immunodeficiency virus (HIV) 1 or 2 antibody.
    g. Diabetes mellitus judged to be in poor control by the Investigator or requiring > 3 anti-diabetic agents counting insulin (all insulins are considered one agent); use of insulin per se is not exclusionary.
    h. History of any primary malignancy with the exception of: curatively treated non-melanomatous skin cancer; curatively treated cervical or breast carcinoma in situ; or other primary tumor treated with curative intent and no known active disease present and no treatment administered during the last 3 years.
9. Undergone major surgery within 6 months of first dose.
10. Current or recent history of psychiatric disorder that in the opinion of the Investigator or Medical Monitor could compromise the ability of the patient to cooperate with study visits and procedures.
11. Use of any of the restricted list of products known to strongly inhibit CYP3A4 drug metabolism (Appendix 15.4, Table 8) within 5 days prior to Day 1 dosing; or to strongly induce CYP3A4

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metabolism (Appendix 15.4, Table 9) within 28 days prior to Day 1 dosing; or to strongly inhibit P-glycoprotein (P-gp) transporter (Appendix 15.4, Table 10) within 5 days prior to Day 1 dosing; or digoxin within 5 days prior to Day 1 dosing. For patients who require chronic inhaled glucocorticoid therapy, Investigators should confer with the Medical Monitor for additional guidance.

12. Serum bilirubin > ULN attributable to factors other than hemolysis and/or Gilbert's syndrome.
13. Male patients with heart-rate corrected QT (Fridericia's correction factor) QTcF interval > 450 msec, or female patients with QTcF interval > 470 msec with the exception of patients with a left bundle branch block (LBBB). Medical Monitor approval needed in patients with a LBBB.
14. Cardiac dysrhythmias judged as clinically significant by the Investigator or requiring therapy with drugs that are primarily substrates of CYP3A4.
15. History of allergy to sulfonamides if characterized by acute hemolytic anemia, anaphylaxis, or rash of erythema multiforme type or Stevens-Johnson syndrome.
16. Any other medical or psychological condition deemed by the Investigator to be likely to interfere with a patient's ability to understand and sign informed consent; cooperate with study visits, tests, and procedures; or otherwise safely and reliably participate in the study.

**Investigational product, dosage, and mode of administration:**
AG-348 sulfate hydrate capsules will be provided as 5 mg, 25 mg, or 100 mg (free-base equivalent) of AG-348 sulfate hydrate without excipients in dark green opaque (5 mg), Swedish orange (25 mg), or white opaque (100 mg) gelatin capsules (size 2 capsules for all dose strengths). AG-348 will be administered orally BID. The number of capsules per dose will vary by assigned dose group. Patients will receive multiple oral (PO) doses of AG-348 over a 24-week treatment period. AG-348 will be administered with water and may be administered with or without food.

**Reference therapy, dosage and mode of administration:**
Not applicable.

**Duration of treatment:**
The duration of treatment for all patients on this study will be up to 24 weeks. Patients who safely tolerate and demonstrate one or more indicators of clinical activity of AG-348 may be eligible to immediately roll over to a safety extension for continued treatment.

**Criteria for evaluation:**

**Safety:**
Monitoring of AEs in randomized patients, including determination of serious adverse events (SAEs) and AEs leading to discontinuation; safety laboratory parameters (hematology, chemistry, urinalysis, coagulation); physical examination findings (including neurological examination); VS; 12-lead ECGs,

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and DXA scans. Adverse events will be graded using CTCAE, Version 4.03. Serum sex hormone levels (testosterone [total and free], estrone, and estradiol), bone turnover markers (serum osteocalcin-N-mid and serum C-terminal telopeptide [CTX]), 25-hydroxy vitamin D2 and D3, total cholesterol, high-density lipoprotein-cholesterol (HDL-C), and triglycerides will be monitored for evidence of potential inhibition of aromatase by AG-348. Menstruating female patients will also keep a paper-based menstrual cycle diary throughout the study.

**Indicators of Clinical Activity:**

Monitoring of potential indicators of clinical activity will include evaluating changes in Hb, HCT, reticulocyte count, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation.

**Pharmacokinetics:**

Approximately the first 10 patients treated, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 15.1, Table 6. The remainder of treated patients will undergo limited PK sampling as detailed in Appendix 15.1, Table 7. Serial blood sampling for determination of concentration-time profiles of AG-348 and its metabolite AGI-8702 will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of AG-348 and AGI-8702 will be obtained. AG-348 and AGI-8702 will be analyzed using qualified assays to determine concentrations in plasma. Pharmacokinetic parameters on Day 1 and Day 15 will be computed using standard non-compartmental methods based on observed plasma AG-348 and AGI-8702 concentrations.

**Pharmacodynamics:**

Pharmacodynamic assessments will include 2,3-DPG, ATP (secondary objectives), [redacted]. Approximately the first 10 patients treated will undergo extensive PD sampling as detailed in Appendix 15.1, Table 6. The remainder of treated patients will undergo limited PD sampling as detailed in Appendix 15.1, Table 7. Serial blood sampling for determination of levels of ATP and, 2,3-DPG will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of ATP and 2,3-DPG will be obtained. Adenosine triphosphate and 2,3 DPG will be analyzed using qualified assays to determine concentrations in whole blood. Pharmacodynamic parameters on Day 1 and Day 15 will be computed based on observed whole blood ATP and 2,3-DPG concentrations.

[redacted]

**Statistical methods:**

The primary objective of this Phase 2 study is to evaluate the safety and tolerability of up to 24 weeks of

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AG-348 in patients with PK deficiency. Therefore, analyses will be primarily descriptive in nature; no formal hypothesis testing will be conducted. All analyses will be conducted separately within each dose arm, or pooled when appropriate.

Summaries will be produced for disposition, baseline disease characteristics and demographic data including genotype, safety measurements, PK, PD parameters and indicators of clinical activity. Data from each AG-348 dose group will be analyzed separately, and pooled across all dose groups where appropriate. All data will also be listed by individual patient. Categorical variables will be summarized by frequency distributions (number and percentages) and continuous variables will be summarized by descriptive statistics (mean, standard deviation [SD], median, minimum, and maximum).

Populations for analysis will include a Safety Analysis Set, a PK Analysis Set, and an Efficacy Analysis Set. The Safety Analysis set will include all patients who are enrolled and receive any dose of study treatment; this population will be the primary set for the analysis of safety data and the default analysis set for all data analyses unless otherwise stated. Patients will be classified according to treatment received, where treatment received is defined as the assigned treatment if it is received at least once, or as the first treatment received if assigned treatment is never received. The PK Analysis Set will include all patients in the Safety Analysis Set with sufficient plasma sample data to assess PK parameters. Results of the potential PD activity of AG-348 will also be based on the PK analysis set. The Efficacy Analysis Set will include all patients who are enrolled and achieve at least 50% compliance with their assigned dose intensity for at least 4 weeks of continued dosing. Additional efficacy analyses may be performed on subsets of patients with other degrees of compliance with their assigned dose intensity and/or duration of dosing. The Efficacy Analysis Set will be the primary set for the analysis of preliminary clinical activity data. Patients will be classified according to assigned treatment. [redacted]

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and the incidence of treatment-emergent AEs (TEAEs) (new or worsening from baseline) will be summarized by primary system organ class (SOC), preferred term (PT), CTCAE Version 4.03 severity, outcome, action taken with study drug, and relationship to study drug by dose group. Separate summaries will be produced for all TEAEs, treatment-related AEs (those considered by the Investigator as at least possibly drug related), SAEs, discontinuations due to AEs, and AEs ≥ Grade 3 severity. Individual patient listings will be provided for any deaths, SAEs, AEs leading to interruption and/or reduction of study drug dose, and AEs leading to discontinuation of treatment. Analyses of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined as any AE with onset beginning at the day of first administration of study drug, throughout the treatment period until 30 days after the last dose of study drug, or any event that was present at baseline but worsened in intensity or was subsequently considered drug-related by the Investigator through the end of the study.

Descriptive statistics will be provided for clinical laboratory values (e.g., hematology, serum chemistry, coagulation studies, urinalysis) and VS data, presented as both actual values and changes from baseline relative to each on-study evaluation. Shift analyses will be conducted for selected laboratory parameters based on the baseline CTCAE grade to maximum CTCAE grade. Where applicable CTCAE terms do

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not exist, a grading system based on the upper and/or lower limits of normal will be used to classify the results.

Electrocardiogram analyses will include individual patient listings and summaries of abnormal and clinically significant ECG results. Actual values and changes from baseline in PR, QRS, QTc intervals will be summarized by visit and dose arm.

Data collected from the menstrual diaries such as the start and stop dates of the menses and the patient reported characteristics of the menses will be presented in a by-patient listing. Additional descriptions of the data may also be performed.

Concomitant medications will be coded using the WHO Drug Dictionary. Concomitant medications prior to and after the start of the study drug will be listed by patient, and summarized by ATC term and dose arm.

Descriptive statistics will be used to summarize PK parameters for the parent compound AG-348 and the metabolite AGI-8702 for each dose group, and where appropriate, for the entire population. Pharmacokinetic parameters will be summarized using the following descriptive statistics: n, mean, standard deviation (SD), coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation.

Descriptive statistics will be used to summarize PD parameters for 2,3-DPG and ATP for each dose group, and where appropriate for the entire population. Pharmacodynamic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation %.

Analyses of serum sex hormones will use appropriate graphic displays and statistical analyses to evaluate patient change from baseline for each parameter. These analyses will include summaries of actual values and change from baseline using appropriate descriptive statistics (mean, SD, median, min and max) as described above.

Analyses evaluating indicators of potential clinical activity of AG-348 in patients with PK deficiency will include changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation (serum iron/iron binding capacity). Characterization of Hb response (e.g., % of patients whose Hb increases by a certain amount), as well as time to Hb response, and duration of Hb response will be explored, among others.

**Interim Review**

No formal statistical analysis will be conducted. Safety data will be reviewed on an ongoing basis by the DRT, who will meet to review safety, PK, PD, and preliminary clinical activity data at regular intervals (approximately every 6 weeks) throughout the duration of the study. The composition of the DRT, its meeting schedule, the data to be reviewed, and the decisions it is empowered to make have been described previously.

Additional interim reviews of data may be conducted to support decision making concerning the current clinical study, the sponsor's development programs in general, or in case of any safety concerns.

**Sample Size**

Due to the rare disease setting, the minimal sample size may be determined by feasibility. Depending on

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| possible early termination of 1 or both of the initial 2 arms or the addition of a 3rd dose arm, the study could enroll up to a maximum of 75 patients. |

# 3. TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

## TABLE OF CONTENTS

## LIST OF TABLES



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# 4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

| Abbreviation or Specialist Term | Explanation |
|---|---|
| 2,3-DPG | 2,3-diphosphoglycerate |
| ACIP | Advisory Committee on Immunization Practices |
| ADME | Absorption, distribution, metabolism, excretion |
| ADP | Adenosine diphosphate |
| AE | Adverse event |
| ALC | Absolute lymphocyte count |
| ALP | Alkaline phosphatase |
| ALT | Alanine aminotransferase |
| ANC | Absolute neutrophil count |
| aPTT | Activated partial thromboplastin time |
| AST | Aspartate aminotransferase |
| ATP | Adenosine triphosphate |
| AUC | Area under the curve |
| $AUC_{0\text{-}12hr}$ | Area under the plasma concentration versus time curve from 0 to 12 hours |
| $AUC_{0\ 24hr}$ | Area under the plasma concentration versus time curve from 0 to 24 hours |
| $AUC_{0\text{-}\infty}$ | Area under the plasma concentration versus time curve from 0 to infinity |
| BCRP | Breast cancer resistance protein |
| BID | Twice-daily |
| BMI | Body mass index |
| BP | Blood pressure |
| BUN | Blood urea nitrogen |
| CDC | Centers for Disease Control and Prevention |
| CFR | Code of Federal Regulations |
| $CL_P$ | Total body plasma clearance |
| $C_{max}$ | Maximum plasma concentration |
| $CO_2$ | Carbon dioxide |
| COHb | Carboxyhemoglobin |
| CTCAE | Common Terminology Criteria for Adverse Events |

| Abbreviation or Specialist Term | Explanation |
|---|---|
| CTX | Serum C-terminal telopeptide |
| CV | Cardiovascular |
| DDI | Drug-drug interaction |
| CYP | Cytochrome P450 |
| DILI | Drug-induced liver injury |
| DLT | Dose-limiting toxicity |
| DRT | Data review team |
| DXA | Dual-energy x-ray absorptiometry |
| ECG | Electrocardiogram |
| ECOG | Eastern Cooperative Oncology Group |
| eCRF | Electronic case report form |
| EDC | Electronic Data Capture |
| EPO | Erythropoietin |
| F | Oral bioavailability |
| FDA | Food and Drug Administration |
| FSH | Follicle stimulating hormone |
| G6PD | Glucose-6-phosphate-dehydrogenase |
| GCP | Good Clinical Practice |
| GFR | Glomerular filtration rate |
| GLP | Good Laboratory Practice |
| Hb | Hemoglobin |
| HBsAg | Hepatitis B surface antigen |
| HCT | Hematocrit |
| HCV | Hepatitis C virus |
| HDL-C | High-density lipoprotein-C |
| HDPE | High density polyethylene |
| hERG | Human ether à-go-go related gene |
| Hib | *Haemophilus influenzae* type b |
| HIV | Human immunodeficiency virus |
| Hp | Haptoglobin |
| $IC_{50}$ | Concentration of drug that achieved half-maximal inhibition |
| ICH | International Conference on Harmonization |

| Abbreviation or Specialist Term | Explanation |
|---|---|
| INR | International normalized ratio |
| IRB | Institutional Review Board |
| IUD | Intrauterine device |
| IV | Intravenous |
| LDH | Lactate dehydrogenase |
| MAD | Multiple ascending dose |
| MCH | Mean corpuscular hemoglobin |
| MCHC | Mean corpuscular hemoglobin concentration |
| MCV | Mean corpuscular volume |
| MedDRA | Medical Dictionary for Regulatory Activities |
| mPKR | PKR mutants |
| MTD | Maximum tolerated dose |
| NCI | National Cancer Institute |
| NOEL | No-observed-effect-level |
| NOAEL | No-observed-adverse-effect-level |
| P-gp | P-glycoprotein |
| PCV13 | Pneumococcal Conjugate |
| PD | Pharmacodynamic |
| PEP | Phosphoenolpyruvate |
| PK | Pharmacokinetic |
| PK deficiency | Pyruvate kinase deficiency |
| PKR | Pyruvate kinase isoform R |
| PO | Oral |
| PPSV23 | Pneumococcal polysaccharide |
| PR | The portion of the ECG wave from the beginning of the P wave to the beginning of the QRS complex |
| PT | Preferred term |
| q12h | Every 12 hours |
| q24h | Every 24 hours |
| QD | Once-daily |
| QRS | QRS interval on an electrocardiogram |
| QTc | Heart-rate corrected QT interval |
| QTcB | Corrected QT interval - Bazett correction formula |

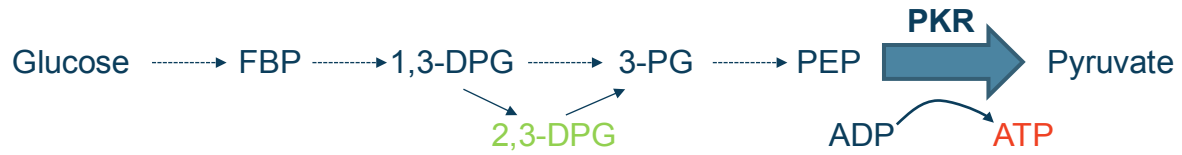
| Abbreviation or Specialist Term | Explanation |
|---|---|
| QTcF | Corrected QT interval - Fridericia's method |
| RBC | Red blood cell (count) |
| RDW | Red cell distribution width |
| SAD | Single ascending dose |
| SAE | Serious adverse event |
| SAP | Statistical analysis plan |
| SD | Standard deviation |
| SOC | System organ class |
| SOP(s) | Standard Operating Procedure(s) |
| $t_{½}$ | Apparent terminal half-life |
| TIBC | Total iron-binding capacity |
| $T_{max}$ | Time to maximum concentration |
| TEAE | Treatment-emergent adverse event |
| ULN | Upper limit of normal |
| VS | Vital signs |
| $V_{ss}$ | Volume of distribution at steady-state |
| Vz/F | Mean apparent volume of distribution |
| WBC | White blood cell |
| WMA | World Medical Association |
| WOCBP | Women of childbearing potential |
| WT | Wild type |

# 5. INTRODUCTION

## 5.1. Pyruvate Kinase Deficiency

Pyruvate kinase deficiency (PK deficiency) is a glycolytic enzymopathy that results in non-spherocytic hemolytic anemia with a variable clinical presentation, ranging from mild or fully compensated forms to life-threatening neonatal anemia and life-long chronic hemolytic anemia associated with severe, debilitating co-morbidities. PK deficiency is caused by mutations in the PKLR gene, which results in defective pyruvate kinase isoform R (PKR) in the red blood cell (RBC). As shown in the diagram below, PKR catalyzes the final and irreversible step in glycolysis, converting phosphoenolpyruvate (PEP) to pyruvate, and formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP). Mature RBCs rely almost exclusively on the process of glycolysis to generate the energy carrier molecule ATP. PKR is thus a key enzyme for maintaining energy homeostasis in erythrocytes, and it has been proposed that ATP levels are critical for optimally maintaining RBC membrane integrity (van Wijk and van Solinge 2005).

**Figure 2: Red Cell Glycolysis**




Abbreviations: 1,3 DPG = 1,3 diphosphoglycerate; 2,3 DPG = 2,3 diphosphoglycerate; 3-PG = 3-phosphoglycerate; ADP = adenosine diphosphate; ATP = adenosine triphosphate; FBP = fructose 1,6-bisphosphate; PEP = phosphoenolpyruvate; PKR = red blood cell-specific form of pyruvate kinase
Note: Not all steps in glycolysis are shown.

The majority of mutations in PKR that have been described have a deleterious effect on PKR catalytic activity, protein stability, and/or protein expression. PK deficiency is associated with reduced RBC survival as well as impaired red cell maturation. Mature erythrocytes lack mitochondria relying predominantly on glycolysis to generate ATP. It is hypothesized that insufficient energy production promotes erythrocyte hemolysis due to impaired maintenance of cellular membrane homeostasis.

PK deficiency is an autosomal recessive disease, in which both homozygotes and compound heterozygotes develop hemolytic anemia. More than 200 different mutations in the PKLR gene have been identified in patients with PK deficiency to date, and the majority of these mutations are single nucleotide missense mutations (Zanella, et al. 2005). The prevalence of PK deficiency, based on the most common pyruvate kinase mutation in Southern Europe, is estimated at 51 cases per million in the Caucasian population (Beutler and Gelbart 2000).

Some patients with PK deficiency have a clinically severe disease course that can present as a severe hemolytic anemia in early infancy, requiring immediate care which typically includes blood transfusion support (Christensen, et al. 2011; Ghidini, et al. 1991; Hennekam, et al. 1990). Infants with PK deficiency may have erythroblastosis fetalis with hepatosplenomegaly, extramedullary hematopoiesis, and severe indirect hyperbilirubinemia, which is associated with a high risk of kernicterus. Hyperbilirubinemia associated with the hemolytic state often requires

supportive treatment with intensive phototherapy. For other infants, the hyperbilirubinemia caused by hemolysis can require exchange transfusion, as well as supportive transfusion to keep their hematocrit (HCT) levels >60%.

Adults with PK deficiency are reported to have life-long hemolysis and the subsequent associated co-morbidities including anemia and transfusion dependence. Other co-morbidities include frequent miscarriages, aplastic crises, as well as symptoms associated with an acute on chronic hemolytic anemia (Rider, et al. 2011). Since unconjugated bilirubin is often chronically elevated, pigmented gallstones are common in children and adults. Severe and sometimes life-threatening iron deposition occurs and is typically progressive.

The current therapeutic options for patients with PK deficiency are supportive. Most require life-long treatment, including blood transfusions at a frequency depending on the disease state. Long-term surveillance for systemic iron overload, even in transfusion-independent patients, is standard as is the use of chelation therapy. Case reports of cure by allogeneic bone marrow transplant have been published (Tanphaichitr, et al. 2000) but have been infrequently performed. Splenectomy, while not curative, can reduce the need for supportive blood transfusions and decrease bilirubin levels in some patients. However, this intervention is associated with significant morbidity including infection risk, and possible increased risk of thrombosis.

## 5.2. AG-348

AG-348 is an orally available, allosteric activator of PKR. It is hypothesized that drug intervention with AG-348 restores glycolytic pathway activity and normalizes red cell metabolism *in vivo*. Biochemical experiments demonstrate that AG-348 is a potent, pan-activator of many PKR alleles associated with PK deficiency. PK deficiency red cells and their progenitors are characterized by changes in metabolism associated with defective glycolysis, including a build-up of PEP and 2,3-diphosphoglycerate (2,3-DPG), and lowered ATP levels. Treatment of PK deficiency patient red cells *ex vivo* with AG-348 results in increased ATP levels, and reductions in PEP and 2,3-DPG, consistent with pharmacological activation of PKR enzyme activity. *In vitro* studies against wild type (WT) PKR enzymes and *ex vivo* studies in blood from mice, monkeys and humans indicate that AG-348 activates WT PKR with a similar potency and efficacy as it does with the mutant versions. *In vivo* studies in mice confirm the potency of AG-348 in increasing WT PKR enzyme activity and modulating the levels of downstream markers such as ATP and 2,3-DPG. This therapeutic approach may be an effective way to correct the underlying pathology of PK deficiency and, importantly, provide clinical benefit to patients.

### 5.2.1. Summary of Nonclinical Data

A series of exploratory pharmacology studies were conducted to characterize the ability of AG-348 to activate WT PKR and anemia-associated PKR mutants (mPKR) *in vitro*, *ex vivo*, and *in vivo*. Additional details regarding nonclinical data can be found in the AG-348 Investigator's Brochure

#### 5.2.1.1. Pharmacology

AG-348 is a potent, broad-spectrum activator of PKR with low nM potency against both WT and mutant enzymes. AG-348 is also able to effectively stabilize mPKR enzymes that are

hypersensitive to thermal denaturation, suggesting that AG-348 may be able to increase PKR pathway activity by preventing destabilization and degradation of mPKR enzymes. Its major metabolite, AGI-8702, has low potency mixed activity against PKR and mutant isoforms and can increase the thermostability of some mPKR, albeit with significantly weaker potency than that of AG-348.

The effect of AG-348 and AGI-8702 on PKR activity and a number of downstream pathway markers was evaluated in human RBCs. AG-348 dose-response curves in human whole blood showed potent activation of PKR, while AGI-8702 is a weak activator of the PKR enzyme. In whole blood from mice treated with AG-348, $^{13}$C-glucose labeling through the PKR reaction was increased by 80% demonstrating AG-348-mediated activation of the WT PKR enzyme. AG-348 treatment of RBCs from healthy human donors potently increased levels of the PKR reaction product ATP consistent with activation of PKR.

The effects of AG-348 on PKR activity and RBC metabolism also were assessed in blood samples from patients with PK deficiency. AG-348 activated PKR and induced metabolic changes (increased ATP levels and decreased 2,3-DPG levels) consistent with increased glycolytic pathway activity in RBCs from PK deficiency patients with different mutations in the PKR enzyme.

Assessments of binding inhibition potential and enzymatic activity were evaluated in a panel of 89 receptors, ion channels, and enzymes. Although AG-348 is a histamine H3 antagonist/inverse agonist and an aromatase inhibitor, no effects of histamine H3 modulation have been observed in safety pharmacology or toxicology studies. Effects consistent with aromatase inhibition were seen in toxicology studies (Section 5.2.1.4).

#### 5.2.1.2. Safety Pharmacology

Safety pharmacology studies demonstrated that neither AG-348 nor AGI-8702 exhibited significant inhibition of the human ether-à-go-go related gene (hERG) current (concentration of drug that achieved half-maximal inhibition [$IC_{50}$] 29.4 µM and > 10 µM, respectively). Additionally, in the monkey cardiovascular (CV) study, no CV effects were seen at any dose level. No effects were seen in AG-348 Good Laboratory Practice (GLP)-compliant Irwin and respiratory safety pharmacology studies in Sprague-Dawley rats. AG-348 administered via oral gavage had a dose-dependent emetic activity in the ferret at ≥ 30 mg/kg.

#### 5.2.1.3. Pharmacokinetics

Absorption, distribution, metabolism, and excretion (ADME) studies of AG-348 were performed in Sprague-Dawley rats, beagle dogs, and cynomolgus monkeys. Both oral (PO) and intravenous (IV) routes were evaluated at doses ranging from 1 to 50 mg/kg. A dose of 200 mg (50 mg/kg) was administered PO in the food-effect evaluation in monkeys. The PK of AG-348 in animal species is characterized by rapid oral absorption, medium to high total body plasma clearance (CLp), and high volume of distribution at steady-state ($V_{ss}$) in rats, dogs, and monkeys.

AG-348 has a medium to long apparent terminal half-life ($t_{1/2}$) in the rat, dog, and monkey. Medium to high oral bioavailability (F) was observed for AG-348 in animals. A high-fat diet led to lower oral absorption of AG-348 in monkeys.

AG-348 showed low brain penetration in rats (brain/plasma ratio of 5.7% to 9.1%) based on area under the plasma concentration versus time curve from 0 to 24 hours ($AUC_{0\ 24hr}$) following single and repeated-dose administration, respectively. Metabolism appears to be the major elimination pathway for AG-348 with minimal biliary or urinary excretion.

Both *in vitro* and *in vivo*, the predominant metabolite was the N-dealkylated product, AGI-8702. There were no metabolites unique to human liver microsomes and no direct Phase 2 metabolites. The plasma metabolite profile of AG-348 did not change with repeat or ascending dosing, suggesting that AG-348 is not susceptible to metabolic switching with increasing or prolonged dose administration.

AG-348 is a weak direct inhibitor of cytochrome P450 (CYP) 2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 (testosterone 6β-hydroxylation). There was no direct inhibition of CYP1A2, CYP2B6, or CYP3A4/5 (midazolam 1′-hydroxylation) by AG-348. There was evidence of metabolism-dependent inhibition of CYP2C19 (largely reversible) and CYP3A4 (largely irreversible) by AG-348. AG-348 is considered a weak time-dependent CYP3A4 inhibitor. AGI-8702 is a weak direct inhibitor of CYP2C9 and CYP2C19. AG-348 has the potential to cause an induction-related drug-drug interaction (DDI) with sensitive CYP2B6 and CYP3A4 substrates.

The routes of metabolism for AG-348 are via multiple CYPs with CYP3A4 contributing > 70% of the total. CYP1A2, CYP2C9, and CYP2C8 contribute approximately 6%, 10%, and 7% to the remaining metabolism of AG-348; other isoforms contribute < 4% each.

AG-348 is a substrate for P-glycoprotein (P-gp), but not breast cancer resistance protein (BCRP). AG-348 is an inhibitor of P-gp (91% and 99% inhibition at 41 and 411 μM, respectively), but does not inhibit BCRP. AGI-8702 is not an inhibitor of P-gp or BCRP under the concentrations tested (5 and 100 μM).

#### 5.2.1.4. Toxicology

In rats, the no-observed-effect level/no-observed-adverse-effect level (NOEL/NOAEL), determined as 2000 mg/kg, was associated with area under the plasma concentration versus time curve from 0 to 12 hours ($AUC_{0\text{-}12hr}$) values 223- to 526-fold the projected human efficacious $AUC_{0\text{-}12hr}$ value. In dogs, clinical observations consistent with anaphylactoid reactions were seen, and the maximum tolerated dose (MTD) was 62.5 mg/kg, which was associated with an $AUC_{0\text{-}12hr}$ value 5.7-fold the projected efficacious $AUC_{0\text{-}12hr}$ value. The NOEL/NOAEL in dogs was 10 mg/kg, which was associated with an $AUC_{0\text{-}12hr}$ value 0.8-fold the projected efficacious $AUC_{0\text{-}12hr}$ value. In monkeys, the NOAEL was 1000 mg/kg, with only non-adverse emesis and body weight loss seen; this dose was associated with an $AUC_{0\text{-}12hr}$ value 70-fold the projected efficacious $AUC_{0\ 12hr}$ value. Based on the results of single-dose studies, the rat and the monkey were chosen as the most appropriate species for further evaluation in toxicology studies.

Dose-limiting toxicity (DLT) in cynomolgus monkeys was defined in non-GLP 5-day and 14-day repeat-dose studies as emesis, inappetence, and weight loss. These toxicities became dose limiting at $AUC_{0\text{-}12hr}$ values 27- to 34-fold the projected efficacious $AUC_{0\text{-}12hr}$ value, and precluded meaningful evaluation of other toxicities at this exposure level. Potential other effects at this exposure level were observed in a number hematology and serum chemistry parameters as well as in lymphoid organs. Additionally, minimal potential effects in kidneys (renal

tubulointerstitial nephritis) and heart (myocardial degeneration), which could not be differentiated from spontaneous background lesions, were seen when monkeys were exposed to AG-348 $AUC_{0\text{-}12hr}$ values ≥ 27- to 34-fold the projected human efficacious $AUC_{0\text{-}12hr}$ value.

In GLP-compliant 28-day monkey study, the dose of 150 mg/kg/day (75 mg/kg/dose twice daily [BID]) was the NOAEL. Effects were limited to increased liver weights without serum chemistry or microscopic correlate. At this dosage level, the Day 27 $AUC_{0\text{-}12hr}$ values were 8.9- and 8.5-fold the projected efficacious $AUC_{0\text{-}12hr}$ value in males and females respectively. In the same study, the low dosage of 20 mg/kg/day (10 mg/kg/dose) resulted in $AUC_{0\text{-}12hr}$ values that approximated the efficacious $AUC_{0\text{-}12hr}$ value, and there were no test article-related effects seen. The next highest dose of 50 mg/kg/day (25 mg/kg/dose) was the NOEL and was associated with $AUC_{0\text{-}12hr}$ values 3.1- and 2.6-fold the projected efficacious $AUC_{0\text{-}12hr}$ value in males and females respectively.

In the GLP-compliant 28-day rat study, the NOAEL in males was the highest dose tested, 600 mg/kg/day (300 mg/kg/dose), and the NOAEL in females was the lowest dose tested, 20 mg/kg/day (10 mg/kg/dose). At the 600 mg/kg/day dosage level in males, AG-348-related findings were limited to mild effects on hematology, serum chemistry, and urinalysis parameters, and microscopic findings in the adrenal gland (minimal to mild vacuolation of the adrenal zona glomerulosa and decreased thickness of the zona fasciculate), liver (minimal to mild hepatocellular hypertrophy), kidney (minimal tubular vacuolation), pancreas (minimal to moderate decreased zymogen granules), heart (minimal myocardial vacuolation), and prostate (minimal to mild decreased secretion). All findings were fully reversible over the 14-day recovery period with the exception of decreased serum glucose levels and decreased prostate secretion. In females, the highest dosage tested was 200 mg/kg/day (100 mg/kg/dose); adverse effects observed were similar to those observed in the 600 mg/kg/day males, with the exception that in females, fewer effects in hematology and serum chemistry parameters were seen, and also in females, adverse effects in the reproductive organs consistent with aromatase inhibition were observed.

In the 13-week repeat-dose rat study, adverse effects in males were identified in the testes and consisted of seminiferous tubular degeneration, spermatid retention, and Leydig cell hypertrophy. These effects were defined as adverse at dose levels at which they are expected to impair fertility. These effects were either not present or present with incidence and severity similar to that of the vehicle group in lower dose levels. Adverse effects in females included uterine atrophy and increased folding of the luminal surface; these effects were defined as adverse at the dose level at which they are expected to impair fertility

In the 13-week repeat-dose study in monkeys, no adverse effects were identified, and no new effects were identified when compared to the 4-week repeat-dose study. Similar to what occurred on the 4-week study, inappetence and emesis during the initial 1-2 weeks of dosing occurred, precluding evaluation of higher doses.

AG-348 inhibits human aromatase activity with an $IC_{50}$ of 2050 nM (based on human placental microsomes) and rat aromatase with an $IC_{50}$ of 493 nM (based on rat ovarian microsomes). In the GLP-compliant 28-day rat study, histologic effects consistent with aromatase inhibition were seen in the female reproductive tract at the mid- and high-dosage levels (100 and 200 mg/kg/day) and included incomplete corpora lutea; ovarian follicular cysts; ovarian cystic, luteinized follicles; uterine atrophy; vaginal mucification; and altered cyclicity. Although these findings

were minimal to mild and were fully reversible (over 14 days), they were considered adverse and the next lower dosage evaluated, 20 mg/kg/day (10 mg/kg/dose BID) was the NOAEL in females. The Day 27 $AUC_{0\text{-}12hr}$ value associated with this dosage level was 6.9-fold the projected human efficacious $AUC_{0\text{-}12hr}$ value. The potential for aromatase inhibition effects occurring in female rats at $AUC_{0\text{-}12hr}$ values > 6.9-fold and < 53-fold the projected efficacious $AUC_{0\text{-}12hr}$ value has been addressed in a 13-week rat study. In this study using doses between the NOAEL and LOAEL in the 28-day study, the NOAEL for histologic lesions of the uterus that may be associated with aromatase inhibition resulted in an $AUC_{0\text{-}12hr}$ that was 26-fold the projected efficacious value. Notably, due to the potency difference of AG-348 against rat versus human aromatase inhibition, there is potential for a 4-fold wider margin for aromatase inhibition in humans versus rats. AGI-8702 is not an aromatase inhibitor.

### 5.2.2. Summary of Clinical Data

To date, 72 healthy adult volunteers have been exposed to AG-348 in 2 clinical studies, a single ascending dose (SAD) study and a multiple ascending dose (MAD) study, with 31 of these subjects exposed to a single AG-348 dose under fasted conditions; 5 exposed to 2 doses of AG-348 under fasted and fed conditions; and 36 exposed to repeated doses of AG-348 for up to 14 days[1]. The following discussion of clinical data refers only to healthy adult volunteer subjects, as this is the first clinical trial in which patients with PK deficiency will be treated with AG-348.

#### 5.2.2.1. Pharmacokinetics

The PK of AG-348 showed low to moderate variability between subjects. After single doses of AG-348 from 30 to 2500 mg, AG-348 was rapidly absorbed as indicated by the short time to maximum plasma concentration ($T_{max}$). There appeared to be some lengthening of the period of absorption at higher doses as indicated by higher $T_{max}$ and a less than proportional increase in maximum concentration ($C_{max}$). Dose-normalized area under the curve (AUC) generally remained constant over the dose range studied, suggesting that AG-348 total exposure increased in a dose-proportional manner. The mean $t_{1/2}$ ranged from 17.8 to 20.4 hours when samples were collected through 72 hours and from 50.3 to 79.3 when samples were collected through 120 hours. However, this terminal elimination phase contributed little to overall exposure of AG-348, as indicated by the small difference between $AUC_{0\text{-}12}$ and area under the plasma concentration versus time curve from 0 to infinity ($AUC_{0\text{-}\infty}$), suggesting a shorter effective half-life of approximately 3 to 6 hours. AG-348 was extensively distributed (mean apparent volume of distribution [$V_z/F$] range of 271 to 1148 L) and had a moderate rate of clearance (geometric mean clearance [CL/F] range of 10.3 to 13.9 L/hr). The fraction of AG-348 excreted in urine ranged from 0.0145 to 0.0209 across the dose levels suggesting that renal excretion plays a minor role in the systemic elimination of AG-348.

The preliminary repeat-dose PK of AG-348 at doses ranging from 15 mg every 12 hours (q12h) to 700 mg q12h also showed an increase in AG-348 exposure in a dose-proportional manner after the first dose. AG-348 exposure observed after repeated dosing for 14 days at doses of

[1] At the time of this document, results from Study AG348-C-002 in healthy volunteers have been unblinded, but the data have only partially been analysed and the Clinical Study Report is in preparation but not yet completed.

120 mg every 24 hours (q24h) to 700 mg q12h were lower than observed on Day 1 after the first dose. The magnitude of the effect was greater at higher doses; lower doses of 15 mg q12h and 60 mg q12h did not show this effect. This observation may be related to the nonclinical finding that AG-348 is a potential inducer of human CYP3A4, an enzyme which biotransforms AG-348. It is possible that multiple doses of AG-348 leads to an increased rate of its own metabolism.

Evaluation of the effect of food on the PK of a single 700 mg dose of AG-348 in 5 subjects who were administered the drug fasting and then, after an appropriate wash-out period, re-administered the drug following ingestion of a standard US Food and Drug Administration (FDA) high fat meal, showed that food likely has a minimal effect on the PK of AG-348.

#### 5.2.2.2. Pharmacodynamics

After a single dose of AG-348, a decrease in the concentration of 2,3-DPG was observed at 3 hours post-dose, decreased in a dose-dependent manner to a minimum at 24-hour post-dose, and then returned to values similar to baseline by 72 to 120 hours post-dose. The mean decrease at 24 hours was approximately 300 µg/mL at the 700 through 2500 mg dose levels. Similar decreases were observed after the first dose of multiple doses and prior to doses in the MAD study. After the final dose of multiple doses, the concentration of 2,3-DPG returned to values similar to baseline between 72 and 120 hours post- dose.

After a single dose of AG-348, a minimal increase in the concentration of ATP was observed at 24 to 120 hours post-dose. In the MAD study, no increase was observed at 12 hours after the first dose; the concentration of ATP was increased on Day 8 to concentrations greater than in the single dose study, continued to trend upward on Day 11, and remained at a similar level through Day 14. The concentration of ATP remained elevated through 120 hours after the last dose on Day 14. The magnitude of the increase in ATP was similar across the dose range from 60 mg BID to 700 mg BID.

#### 5.2.2.3. Safety

Overall, AG-348 has been well-tolerated among healthy volunteers at doses that produced strong pharmacodynamic effects on 2,3-DPG and ATP.

After a single AG-348 dose, treatment-emergent adverse events (TEAEs) reported by > 1 subject at any time on study (either under fasted or fed conditions) included headache (22%), nausea (14%), and contact dermatitis and vomiting (each 6%). After repeated dosing of AG-348 for 14 days, TEAEs that occurred in > 5% of all AG-348-treated subjects across all cohorts included headache (13.9%), nausea (13.9%), vomiting (8.3%), decreased appetite (8.3%), feeling hot (8.3%), restlessness (8.3%), and dizziness, fatigue, vessel puncture site bruise, hyperhidrosis, dermatitis allergic, and drug eruption (5.6% each).

A dose-relationship was apparent with regard to the incidence of gastrointestinal events, primarily nausea and vomiting, with the incidence of such events increasing with increasing dose. Nausea and/or vomiting were observed only at doses ≥ 1400 mg in the single dose study and only at doses ≥ 700 BID in the MAD study. Nausea and vomiting were not observed at any dose ≤ 360 mg in either the single or multiple dose studies.

All but 1 TEAE reported to date has been mild or moderate (Grade 1 or 2) in intensity. The only Grade 3 TEAE was elevated liver transaminases (alanine aminotransferase [ALT]; aspartate

aminotransferase [AST]) in a single subject in the MAD study treated with 700 mg AG-348 BID. The event was considered to be possibly related to AG-348, was declared a DLT, and led to study drug discontinuation, following which the elevated liver transaminases resolved. Altogether, 3 of 6 subjects treated with AG-348 at 700 mg BID discontinued study drug in the MAD study: the subject described above with Grade 3 elevated transaminases, and 2 others who withdrew themselves for nausea and vomiting. A fourth subject treated with AG-348 at 60 mg BID was discontinued from the study for a drug eruption. No AG-348-treated subject discontinued in the SAD study due to an adverse event (AE).

No deaths or other serious adverse events (SAEs) have been reported in any clinical study of AG-348. Furthermore, no DLTs were observed after a single administration of AG-348 at doses of 30 mg to 2500 mg or after repeat administration of AG-348 for 14 days at daily doses of 15 mg to 700 mg BID except for the event of Grade 3 elevated liver function tests described above.

Due to preclinical observations pertaining to the potential for inhibition of the aromatase enzyme (see Section 5.2.1.4), the AG348-C-002 multiple dose study included assessment of baseline and serial measures of free and total serum testosterone and serum estradiol and estrone. The unblinded serum hormone data are undergoing analysis, but at least some male subjects treated with all doses of AG-348 demonstrated modest increases in androgenic hormones and decreases in estrogens, compatible with a potential signal of aromatase inhibition, whereas the males who received placebo did not. These changes were reversible upon cessation of dosing. There were too few female subjects in the study who received AG-348 to permit any definitive conclusions. Additional analyses are ongoing.

## 5.3. Study Rationale

Study AG348-C-003 is the first study that will be conducted in patients with PK deficiency. This study is primarily intended to evaluate the safety and tolerability and potential indicators of clinical activity of AG-348 administered for up to 24 weeks. This study will also evaluate the PK profile of AG-348 and its metabolite AGI-8702, the PD responses in ATP and 2,3-DPG following administration of AG-348, and the clinical activity of AG-348 in PK deficiency patients. Two previous double-blind, placebo-controlled clinical trials of AG-348 conducted in healthy adult male and female volunteers (AG348-C-001, a SAD study; and AG348-C-002, a MAD study) have established an acceptable safety and tolerability profile for AG-348 for up to 14 days of both once-daily (QD) and BID dosing at exposures that result in significant pharmacodynamic (PD) changes in whole blood levels of the glycolytic metabolites 2,3-DPG and ATP. Due to the potential for AG-348-mediated aromatase inhibition, dual-energy x-ray absorptiometry (DXA) scan (hip and spine) will be performed at Screening (if patient has not had prior DXA scan within 3 months of Day 1) to obtain T and Z scores. These data will serve as baseline measures of bone mineral density for all enrolling patients (see Section 7.1 for more details).

The target population of this study consists of adult males and females with a diagnosis of PK deficiency, who are anemic but non-transfusion dependent. Non-transfusion dependent patients are preferred for this study in order to reduce any potential confounding effect of transfusion therapies on evaluation of potential indicators of clinical activity and PD responses. The safety,

tolerability, and PK/PD findings in this study will form the basis for subsequent clinical development of AG-348.

### 5.3.1. Summary of Overall Safety Management Plan

Measures to minimize the risks to patients enrolled in this study have been taken with respect to the following study design elements:

- The initial doses for Arms 1 and 2 have been selected on the basis of safety and tolerability observations already carefully made in two predecessor clinical trials in adult healthy male and female volunteers;
- The specified inclusion/exclusion criteria have been carefully considered to avoid enrollment of subjects for whom exposure to the study drug might pose a hazard;
- A designated Data Review Team (DRT) consisting of the overall study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer has been established to meet regularly at approximately 6-week intervals throughout the duration of the study to review the accumulating study data and will exercise options to suspend enrollment to one or both of the initial two study dose arms, discontinue enrollment to one or both of the initial two study dose arms, adjust the dose of patients in one or both of the initial two study arms, and/or implement one new study dose arm. If one new dosing arm is implemented, the dose selected will not exceed 360 mg BID, the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy volunteers. Group cohort stopping rules for terminating enrollment into an arm based on the severity (CTCAEv4.03 grade) and frequency of AEs are defined;
- Dose modification and stopping rules are defined for individual patients;
- Guidance for permitted, prohibited, and cautionary concomitant medications is specified based on the estimated potential for drug-drug interactions from hepatic cytochrome enzyme interactions with AG-348.
- Due to the potential for AG-348-mediated aromatase inhibition, bone mineral density will be monitored using DXA scans (hip and spine) at Baseline (if patient has not had prior DXA scan within 3 months of Day 1) and between Week 24 and Week 28.

In the event that any clear and unequivocal, previously unidentified/unexpected toxicities occur in pre-clinical toxicology studies, the Sponsor will notify the Investigators, IRBs/ECs, and regulatory authorities. The Sponsor will also report these to the DRT for review and discussion of appropriate actions, which may include, but are not necessarily limited to:

- Amending the protocol to adjust the inclusion/exclusion criteria (e.g., to exclude patients with certain at-risk concurrent conditions); and/or
- Amending the protocol to adjust safety monitoring procedures (e.g., to require additional monitoring of specified adverse events, physical examinations, clinical laboratory testing, ECG monitoring, or other testing as appropriate); and/or
- Adjusting the dose of an arm of the study as appropriate; and/or

- Adjusting the dose modification and/or stopping rules (Section 9.7); and/or
- Adjusting the patient withdrawal criteria (Section 8.6); or
- Terminating the trial.

# 6. TRIAL OBJECTIVES AND ENDPOINTS

## 6.1. Primary Objective

The primary objective of the study is to:

- Evaluate the safety and tolerability of up to 24 weeks of AG-348 administration in patients with PK deficiency.

## 6.2. Secondary Objectives

The secondary objectives of the study are to:

- Evaluate the pharmacokinetics (PK) of AG-348 and the metabolite AGI-8702.
- Evaluate the PD response of ATP and 2,3-DPG after administration of AG-348.
- Evaluate indicators of clinical activity of AG-348 in patients with PK deficiency, including changes in hemoglobin (Hb), HCT, reticulocyte count, haptoglobin (Hp), carboxyhemoglobin (COHb), lactate dehydrogenase (LDH), total and indirect bilirubin, erythropoietin (EPO), ferritin, and transferrin saturation (serum iron/iron binding capacity).

## 6.4. Study Measures and Endpoints

### 6.4.1. Safety Measures and Endpoints

Safety will be evaluated by:

- Monitoring of AEs, including determination of SAEs and AEs leading to discontinuation; safety laboratory parameters (hematology, chemistry, urinalysis, coagulation); physical examination findings; vital signs (VS); 12 lead electrocardiograms (ECGs); and DXA scans. Adverse events will be graded using Common Toxicity Criteria for Adverse Events (CTCAE), Version 4.03. Serum sex hormone levels (testosterone [total and free], estrone, and estradiol), bone turnover markers (serum osteocalcin-N-mid and serum C-terminal telopeptide [CTX]), 25-hydroxy vitamin D2 and D3, total cholesterol, high-density lipoprotein-cholesterol (HDL-C), and triglycerides will be monitored for evidence of potential inhibition of

aromatase by AG-348. Menstruating female patients will also keep a paper-based menstrual cycle diary throughout the study.

### 6.4.2. Clinical Activity Measures and Endpoints

- Monitoring of potential indicators of clinical activity will include evaluating changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation.

### 6.4.3. Pharmacokinetic and Pharmacodynamic Measures and Endpoints

The PK and PD profile of AG-348 will be evaluated by:

- Approximately the first 10 patients treated, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 15.1, Table 6. The remainder of treated patients will undergo limited PK sampling as detailed in Appendix 15.1, Table 7. Serial blood sampling for determination of concentration-time profiles of AG-348 and its metabolite AGI-8702 will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of AG-348 and AGI-8702 will be obtained. AG-348 and AGI-8702 will be analyzed using qualified assays to determine concentrations in plasma. Pharmacokinetic parameters on Day 1 and Day 15 will be computed using standard non-compartmental methods based on observed plasma AG-348 and AGI-8702 concentrations.
- Pharmacodynamic assessments will include 2,3-DPG, ATP (secondary objectives), [redacted] Approximately the first 10 patients treated will undergo extensive PD sampling as detailed in Appendix 15.1, Table 6. The remainder of treated patients will undergo limited PD sampling as detailed in Appendix 15.1, Table 7. Serial blood sampling for determination of levels of ATP and, 2,3-DPG will be conducted following the first dose and the morning Day15 dose, and additional trough levels of ATP and 2,3-DPG will be obtained. ATP and 2,3 DPG will be analyzed using qualified assays to determine concentrations in whole blood. PD parameters on Day 1 and Day 15 will be computed based on observed whole blood ATP and 2,3-DPG concentrations.

[redacted]

# 7. INVESTIGATIONAL PLAN

## 7.1. Overall Study Design

Study AG348-C-003 is a Phase 2, open-label, two-arm, multicenter, randomized, dose-ranging study during which adult patients with PK deficiency will receive multiple doses of AG-348 for up to 24 weeks. Patients with PK deficiency confirmed by red blood cell PK enzymatic assay performed at Screening will be eligible to participate in this study. At Week 25, patients who safely tolerate AG-348 and demonstrate clinical activity of AG-348 may be eligible to immediately roll over to a safety extension for continued treatment. Patients who finish treatment at the end of 24 weeks or sooner will undergo follow-up assessment 4 weeks after the last dose of study drug. Patients with toxicity suspected to be related to study drug will continue follow-up until the AE resolves, is declared chronic by the Investigator, or the patient is lost to follow-up.

Initially, up to 25 patients will be randomized on an open-label, 1:1 basis to each of two BID doses of AG-348 (up to 50 patients; see Figure 3, Study Schema). The dose of Arm 1 is 300 mg of AG-348 administered orally q12h (BID). The dose of Arm 2 is 50 mg of AG-348 administered orally q12h (BID). Randomization will be stratified by *PKR* mutation in order to maintain balance as much as possible across the dose arms for the specific mutations expected to be most frequently enrolled. The *PKR* mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H) and all other mutations ("other"). Mutation status is defined by the presence of at least one of the indicated mutations; patients with more than one stratified mutation will be assigned based on Sponsor's discretion.

The doses for each arm have been selected from the forerunner AG348-C-001 SAD study and AG348-C-002 MAD studies in healthy adult volunteers to represent the range of doses/exposures that were safely tolerated and resulted in maximal or near maximal PD effects on 2,3-DPG and ATP.

Because PK deficiency is a rare disease with a limited eligible patient population and because the underlying pathophysiology and clinical phenotype of affected patients is heterogeneous due to the wide variety of mutations in PKR that cause the disease, it is deemed important to focus closely on dose findings in this first-in-patient study. Therefore, in addition to initiating this study with 2 different doses of AG-348 administered q12h, a DRT will be established to review study data on a frequent basis and adapt the study design, dose and schedule of AG-348 as indicated.

The DRT will monitor safety on an on-going basis and meet at regular intervals (approximately every 6 weeks), or *ad hoc* as necessary, to review AEs, VS, clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and ECGs on enrolled patients. The DRT will also review available PK/PD data and indicators of clinical activity (e.g., changes from baseline in Hb). The DRT will be comprised of the study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer.

Approximately every 6 weeks, beginning 6 weeks after the first patient is doses or *ad* hoc as necessary, the DRT will review cumulative safety data, available PK/PD data, and clinical

activity data. Based on the DRT's recurring 6-week reviews, the DRT may exercise one or more of the following options:

- Continue treatment and enrollment in existing arms without change.
- Add 1 new dose arm (Arm 3) to enroll up to 25 patients at a dose to be determined; the dose for Arm 3 may be lower or higher than Arm 1 and Arm 2 doses, but will not exceed 360 mg q12h; and the dose regimen may be less frequent than q12h.
- Terminate or suspend enrollment to allow further review of clinical data in Arm 1 and/or Arm 2 (and/or potential Arm 3). Enrollment in an arm could be terminated or suspended to allow further review, for example, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity.
- Re-assign patient's doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the patients in the terminated arm will remain in their original arm, i.e., they will not count towards the enrollment quota of the arm whose dose and schedule is being adopted.
- Implement specific genotype restrictions to enrollment in one or more arms to ensure representation of patients with genotypes of greatest clinical relevance.

The DRT will perform these evaluations on a recurring 6-week basis. The data that the DRT will review to make these decisions is expected to include, but are not necessarily limited to, the following:

- *Safety Observations:* all AEs; VS, clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and ECGs;
- *PK and PD Observations:* including changes in 2,3-DPG and ATP;
- *Indicators of Clinical Activity:* including changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, EPO, total and indirect bilirubin, ferritin, and transferrin saturation.

If a third dose arm is implemented, the dose of AG-348 selected will not exceed 360 mg BID, as this was the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy volunteers. The PK/PD sampling schedule in a potential third dosing arm will be determined by Sponsor's discretion and may follow either the extensive or limited PK/PD sampling schedules as specified.

The DRT will monitor the safety data in an ongoing manner as described and may make a decision to terminate enrollment in an arm if unacceptable AEs are observed, PD activity is limited, and/or markers of clinical activity indicate no effect. Unacceptable AEs are defined as ≥ Grade 3 AEs (using National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAEv4.03]) that are assessed as at least possibly related to AG-348, with the exception of Grade 3 headache, hot flash/flushing, nausea, vomiting, and/or diarrhea that is transient in duration (< 24 hours) or able to be medically managed to ≤ Grade 2 within 24 hours. The DRT may also exercise discretion to terminate enrollment in an arm if it observes a frequency of Grade 2 AEs that would make it difficult to support long-term dosing.

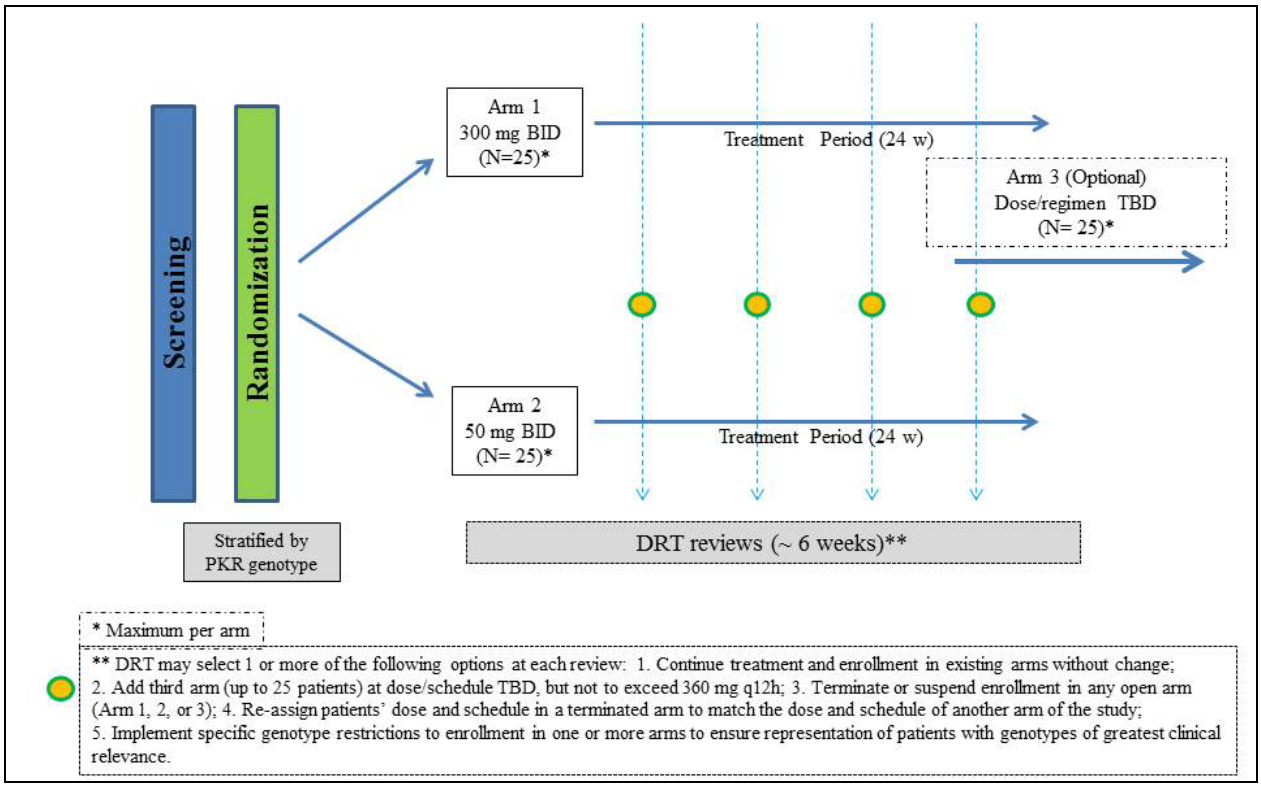
Due to the potential for AG-348-mediated aromatase inhibition, DXA scan (hip and spine) will be performed at Screening (if patient has not had prior DXA scan within 3 months of Day 1) to

obtain T and Z scores. These data are intended to serve as a baseline measure of bone mineral density for all enrolling patients, and are deemed of particular importance for those who may enter the longer term safety extension after completing 24 weeks of treatment. All patients will have a second DXA scan in the interval between Weeks 24 and 28. The safety extension will address additional follow-up DXA scanning.

As the number of enrolling arms changes in the study (for example, from 2 to 3), the randomization scheme will adjust to enable balanced randomization into each actively accruing arm. Randomization and stratification will cease in the event that only a single arm is left enrolling.

Depending on possible early termination of 1 or both of the initial 2 arms, or the addition of a third arm, the study could enroll up to a maximum of 75 patients.

**Figure 3: Study Schema**




Abbreviations: BID (q12h) = twice-daily (every 12 hours); DRT = data review team; PKR = pyruvate kinase red blood cell isoform; TBD = to be determined; w = weeks.

The Investigator will monitor all patients for safety and tolerability. Modification of an individual patient's dose of AG-348 will be based on AEs and observed changes in Hb levels as detailed in Section 9.7.1 and Section 9.7.2.

Screening assessments will occur within 28 days prior to the first dose of study treatment. During the Treatment period, patients will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12), and monthly through Week 24 (Weeks 16, 20, and 24). Patients who safely tolerate AG 348 and demonstrate evidence of clinical activity of AG-348 through Week 24 may be eligible to

immediately enter a safety extension for continued treatment. For patients who finish treatment at the end of 24 weeks or sooner, or who elect not to enter the safety extension, study discharge will occur 4 weeks (Week 28 or earlier) following the last dose of study treatment at the final follow-up assessment.

Safety assessments will include monitoring of AEs, including determination of SAEs and AEs leading to discontinuation; safety laboratory parameters (e.g., hematology, serum chemistry, coagulation studies, and urinalysis); physical examination findings; VS; 12 lead ECGs, and DXA scans. Additional safety assessments will include monitoring of sex hormone levels (testosterone [total and free], estrone, and estradiol), bone turnover markers (osteocalcin-N-mid and CTX), 25-hydroxy vitamin D2 and D3 levels, total cholesterol, high-density lipoprotein-cholesterol (HDL-C), and triglycerides. Follow-up assessments will be conducted on Day 197 (Week 28) and will include physical examination, weight, performance status, VS, 12-lead ECGs, laboratory evaluations (hematology, Hp, EPO levels, serum chemistry, coagulation studies, urinalysis; lipids, hormonal testing), AEs, and transfusion record. Menstruating female patients will also be required to keep a paper-based menstrual cycle diary throughout the study.

Pharmacokinetic assessments will include serial blood sampling for PK profiles of AG-348 and its metabolite AGI-8702. Pharmacodynamic evaluations will include serial blood sampling for determination of levels of ATP and 2,3 DPG. Extensive PK/PD sampling will be conducted on the first approximately 10 patients total treated in Arms 1 and 2 (see Appendix 15.1, Table 6) while more limited PK/PD sampling will be conducted on additional patients treated if enrollment in Arm 1 or Arm 2 is expanded or if an alternate dosing arm is added (see Appendix 15.1, Table 7).

## 7.2. Justification of the Study Design

The primary and secondary objectives of this study are to evaluate the safety, tolerability, PK and PD, and indicators of clinical activity of AG-348 in patients with PK deficiency. The choice of dose and schedule of administration of AG -348 for Arms 1 and 2 was based on the highest safely tolerated dose (Arm 1: 300 mg BID) and the lowest dose with potentially relevant PD activity (Arm 2: 50 mg BID) from the forerunner AG348-C-002 MAD study in healthy volunteers. Decisions regarding continuing enrollment and treatment in these initial dose arms and/or implementation of an additional dose arm will be based on DRT review of safety, PK and PD data, and indicators of clinical activity collected from all patients treated in Arm 1 and Arm 2. This design was chosen to minimize risk to patients while allowing evaluation of safe and pharmacologically active dose levels of AG-348, and to allow the necessary flexibility to adjust dose and schedule should the safety, tolerability, PK, and/or PD be different in patients with PK deficiency compared with healthy volunteers.

Additional safety measures intended to minimize risk to patients include monitoring of AEs by the DRT and specified provisions for individual patient dose modification as needed for safety and (potentially) large increases in Hb level (Section 9.7.1 and Section 9.7.2). Measures intended

to maximize the opportunity for patients with demonstrated safety and tolerability to continue to derive benefit from any observed clinical activity of AG-348 include the option for continued treatment on a safety extension.

A comprehensive series of safety evaluations, including laboratory parameters, physical examinations, VS, 12-lead ECGs, and monitoring for SAEs and AEs, will be conducted to evaluate the safety profile of AG-348 and to aid in the determination of the recommended dose for continued development.

The study includes serial blood sampling across at least two different doses of the study drug to assess its PK and PD profiles.

Consistent with the design of many Phase 2 studies, preliminary evaluation of the potential PD and clinical activity of AG-348 are secondary objectives of this study. The latter will include assessments of the PK/PD relationship between AG-348 and the biomarkers ATP and 2,3-DPG, [redacted]

## 7.3. Rationale for the Starting Dose, Dose Range, and Duration of Dosing

Prior to execution of this study, Agios conducted two clinical studies of AG-348 in healthy volunteers, including a SAD study (AG48-C-001) and a MAD (14 day q12h) study (AG348-C-002). Available details of these studies are discussed in the current Investigator's Brochure (IB). Between these two studies, 72 healthy human subjects have been dosed with AG-348. *In vitro* investigations, also reported in the IB, had previously demonstrated that AG-348 increased the activity of wild-type PKR approximately to the same extent as it did a series a recombinant mPKRs. Therefore it was deemed reasonable to study the safety, tolerability, PK, and PD of AG-348 in healthy subjects in a controlled Phase 1 setting as a more efficient means of obtaining information than in the actual rare disease population of patients with PK deficiency.

The MAD study demonstrated that the exposures produced by AG-348 doses from 60 mg q12h to 360 mg q12h (including 120 mg q24h) resulted in maximal changes from baseline for the PD markers 2,3-DPG (reduction from baseline) and ATP (increase from baseline). The exposures resulting from doses less than 60 mg q12h were of lesser magnitude and the exposures resulting from doses greater than 360 mg q12h were of no greater magnitude than the aforementioned range. Therefore the starting doses for this first dose ranging study in patients with PK deficiency were selected to be 300 mg q12h (Arm 1) and 50 mg q12h (Arm 2). These doses were demonstrated to be safe and tolerable in the healthy volunteer studies. The availability of ATP is proposed as being critical for optimally maintaining RBC membrane integrity (see Section 5.1). The dose ranges from 50 mg q12h to 300 mg q12h may result in clinically effective modulation of PKR in PK deficiency patients if the mutated enzyme is responsive to AG-348 in a similar manner to the wild-type enzyme in healthy subjects. However, there are many different mutations in PKR that result in PK deficiency, and these mutations produce variable effects on the enzyme in terms of catalytic activity and thermal stability. It is not known if different mutations will respond clinically in a similar manner to the same exposure to AG-348. Therefore, it is prudent to study the range of safe and pharmacodynamically relevant doses as specified in this study, and to allow flexibility for the DRT to analyze the evolving study data to adapt the dose and schedule of administration of AG-348 to produce the optimal combination of safety, tolerability, and PD, and, potentially, clinical response.

**Justification for Duration of Dosing**

The treatment duration of 24 weeks (6 months) was chosen for this proof-of-concept trial for 2 principal reasons: 1) to begin establishing a safety database addressing the chronic administration of AG-348; and 2) to allow sufficient time for clinical response to treatment to appear.

It is anticipated that this treatment, if successful, may be taken for life, as PK deficiency is a genetically determined inborn error of metabolism. Therefore, it is important to begin to investigate the long-term safety of the treatment. As will be discussed below, the safety package supports the treatment duration of 6 months.

Red blood cell turnover is typically 120 days (4 months), although it may be shorter in some populations of pyruvate kinase deficient RBCs (Mentzer, et al. 1971). Different PKR mutations produce a variety of physiologic consequences. In mutations where normal or nearly normal levels of PKR protein persist, but the protein's catalytic function is impaired, an improvement in PKR functional activity might be seen relatively quickly upon exposure to AG-348. However, in cases where the PKR mutation results in an unstable mutant, PKR protein levels may be low and additional time may be required for stabilization of the newly synthesized mutant enzyme in developing bone marrow erythroblasts so that eventually more mature RBCs may be produced with more nearly normal levels of functional PKR protein.

This study plans up to 24 weeks of dosing with AG-348 with the possibility of continued dosing beyond 24 weeks on a safety extension in patients for whom AG-348 is safely tolerated and demonstrates clinical activity. The ICH Guideline M3(R2) on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorisation for Pharmaceuticals, recommends that clinical trials of a duration between 2 weeks and 6 months should be supported by repeat-dose toxicity studies of at least equivalent duration, and clinical trials lasting beyond 6 months should be supported by 6-month rodent and 9-month non-rodent studies. This guidance allows for clinical trials of longer duration to be initiated based on 3 months of nonclinical data, and for clinical dosing in serious or life-threatening indications to be extended based on complete chronic rodent data and in-life and necropsy data in the non-rodent (and complete histopathology in the non-rodent provided within an additional 3 months).

For the current investigational product (AG-348), 13-week, repeat dose toxicology studies in the rat and monkey have been completed and are summarized in Section 5.2.1.4 of this protocol and in the current Investigator Brochure prepared to support initiation of this clinical study. Considering that PK deficiency is a serious condition with an unmet medical need, it is appropriate to initiate this clinical study with the available nonclinical data. Six-month rodent and nine-month monkey toxicology studies were initiated in January 2015, and the Sponsor will report the results of these studies in each applicable regulatory region as required.

## 7.4. Criteria for Study Termination

This study may be prematurely terminated if, in the opinion of the Sponsor, there is sufficient reasonable cause. In the event of such action, written notification documenting the reason for study termination will be provided to each Investigator.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to patients;
- Insufficient adherence to protocol requirements;
- Plans to modify, suspend, or discontinue the development of the study drug;
- Other administrative reasons.

Should the study be closed prematurely, all study materials must be returned to the Sponsor or the Sponsor's designee.

# 8. STUDY POPULATION

## 8.1. Number of Patients

Up to approximately 75 patients may be enrolled in this study.

## 8.2. Inclusion Criteria

For entry into the study, patients must meet all of the following criteria during the Screening or other specified period:

1. Signed written informed consent obtained prior to performing any study procedure, including screening procedures.
2. Male or female, aged 18 years and older.
3. Known medical history of PK deficiency.
4. All patients must have documented clinical laboratory confirmation of PK deficiency by RBC pyruvate kinase enzymatic assay performed at Screening by a designated central laboratory. Patients with prior documentation of PK deficiency by RBC enzymatic assay will have a reconfirmation of this result during Screening as a condition of enrollment.
    a. In the event that a patient's screening pyruvate kinase enzymatic assay is negative (i.e., shows normal pyruvate kinase activity), the patient will be eligible for enrollment if the genotyping shows a mutant genotype that has been previously documented in the literature to be associated with pyruvate kinase deficiency. If the genotyping shows a previously undescribed mutation in the PKR gene, then the eligibility for enrollment will be determined on a case-by-case basis by the Coordinating Investigator and Medical Monitor in discussion with the Investigator. If no mutation is defined, then the patient will not be eligible.
5. All patients must have genotypic characterization of the mutant PKR gene performed by a designated central laboratory at Screening, unless genotype is available from the patient's participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480).
6. All patients must have genotypic characterization of the UGT1A1 gene performed by a designated central laboratory to document whether they may have underlying Gilbert's Disease. Patients with Gilbert's Disease are eligible to enroll.
7. Males must have Hb $\leq$ 12.0 g/dL; females must have Hb $\leq$ 11.0 g/dL.
8. All patients must be considered transfusion independent as defined by: no greater than 3 units of RBCs transfused in the 12-month period up to the first day of study dosing and no transfusions within 4 months of first day of study dosing. Patients who have received more transfusion support than described above will evaluated for eligibility on a case-by-case basis by the Medical Monitor.
9. Eligible patients may still have their spleens in place, or may have undergone prior splenectomy. For splenectomized patients:

a. Must have undergone their procedure at least 6 months prior to Screening.

b. Must be current in their vaccinations for Pneumococcal Conjugate (PCV13), Pneumococcal Polysaccharide (PPSV23), Quadrivalent Meningococcal vaccine, and Haemophilus influenzae type b (Hib) as recommended by Centers for Disease Control and Prevention's (CDC) Advisory Committee on Immunization Practices (ACIP) or immunization advisory groups in Canada and the European Union (for patients enrolled in Canada and the EU). [http://www.cdc.gov/vaccines/schedules/downloads/adult/adult-schedule.pdf] [Any missing vaccinations may be administered during the screening period.]

10. Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 2 (Appendix 15.2).
11. Patients must be taking at least 1 mg of folic acid daily for at least 21 days prior to first dose and continued daily during study participation.
12. Adequate organ function, defined as:

    a. Serum AST and ALT ≤ 1.5 × upper limit of normal (ULN) (unless the increased AST is assessed by the Investigator as due to hemolysis).

    b. Normal or elevated levels of serum bilirubin. In patients with serum bilirubin > ULN, the elevation must be attributed to hemolysis with or without Gilbert's syndrome and must not be choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease.

    c. Serum creatinine ≤ 1.25 × ULN. If serum creatinine > 1.25 × ULN, then 24-hour measured or calculated (Cockcroft-Gault) glomerular filtration rate (GFR) ≥ 60 mL/min.

    d. Absolute neutrophil count (ANC) > 1.0 × 109/L.

    e. Platelet count ≥ 100 × 109/L.

    f. Activated partial thromboplastin time (aPTT) and international normalized ratio (INR) ≤ 1.25 × ULN, unless the patient is receiving therapeutic anticoagulants.

13. Women of childbearing potential (WOCBP) must agree to abstain from sexual intercourse or to use an acceptable/effective method of contraception (i.e., condom plus spermicide, condom plus oral contraceptive, condom plus intrauterine device [IUD], condom plus diaphragm with spermicide) from as soon as feasible during the Screening period until 30 days following the last dose of AG-348. Abstinence is an acceptable method only when this is in line with the normal life style of the patient, meaning that the patient plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence, e.g. calendar, sympathothermal and post-ovulation methods, and withdrawal are not acceptable methods of contraception.

    a. WOCBP include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, and/or bilateral oophorectomy) or is not post-menopausal. Post-menopausal is defined as:

i. Amenorrhea ≥ 12 consecutive months without another cause, and a documented serum follicle stimulating hormone (FSH) level > 35 mIU/mL;

ii. Amenorrhea≥ 12 consecutive months in women ≥ 62 years old (FSH testing is not required).

14. WOCBP must have a negative serum or urine pregnancy test within 72 hours before start of AG-348 dosing.
15. Women must not be breastfeeding.
16. Male patients, with the exception of those who have undergone vasectomy at least 6 months prior to Screening, must agree to abstain from sexual intercourse or, if sexually active, to use a condom with spermicide as contraception (regardless of their female partner's childbearing potential or their partner's use of their own contraception) from Day 1 of dosing until 30 days following the last dose of AG-348. Abstinence is an acceptable method only when this is in line with the normal life style of the patient, meaning that the patient plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence, e.g. selective timing of intercourse based on partner's calendar, sympathothermal and post-ovulation methods, and withdrawal are not acceptable methods of contraception.

## 8.3. Exclusion Criteria

Patients who meet any of the following criteria at Screening or prior to dosing on Day 1 will not be enrolled in the study:

1. Hemoglobin level > 12.0 g/dL if male; Hb > 11.0 g/dL if female.
2. Additional diagnosis of any other congenital or acquired blood disorder, including glucose-6-phosphate-dehydrogenase (G6PD) deficiency, or any other hemolytic anemia process except for mild allo-immunization as a consequence of transfusion therapy.
3. Iron overload (hemosiderosis or concurrent hemochromatosis) sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac, hepatic, or pancreatic insufficiency.
4. Prior bone marrow or stem cell transplant.
5. Clinically symptomatic cholelithiasis or cholecystitis. (Prior cholecystectomy is not exclusionary. Patients with symptomatic cholelithiasis or cholecystitis may be rescreened once the disorder has been treated and clinical symptoms have resolved.)
6. Currently enrolled in another therapeutic clinical trial involving on-going therapy with any investigational or marketed product or placebo. Concurrent participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480) is permitted.
7. Exposure to any investigational drug, device, or procedure within 28 days prior to Screening.

8. Concurrent medical condition that could compromise participation in the study such as:
    a. Poorly controlled hypertension (defined as systolic blood pressure (BP) > 150 mm Hg or diastolic BP > 90 mm Hg) refractory to medical management.
    b. History of recent (within < 6 months from Screening date) congestive heart failure; myocardial infarction or unstable angina pectoris; or hemorrhagic, embolic, or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.
    c. Currently active infection requiring the use of parenteral anti-microbial agents or that is ≥ Grade 3 (CTCAEv4.03) within 6 months of first dose.
    d. A pattern or frequency of post-splenectomy sepsis that in the assessment of the Investigator could reasonably be expected to interfere with the ability of the patient to complete the 24 week study participation.
    e. Positive test for hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV) antibody with signs of active Hepatitis B or C virus infection.
    f. Positive test for human immunodeficiency virus (HIV) 1 or 2 antibody.
    g. Diabetes mellitus judged to be in poor control by the Investigator or requiring > 3 anti-diabetic agents counting insulin; use of insulin *per se* is not exclusionary.
    h. History of any primary malignancy with the exception of: curatively treated non-melanomatous skin cancer; curatively treated cervical or breast carcinoma *in situ*; or other primary tumor treated with curative intent and no known active disease present and no treatment administered during the last 3 years.
9. Undergone major surgery within 6 months of first dose.
10. Current or recent history of psychiatric disorder that in the opinion of the Investigator or Medical Monitor could compromise the ability of the patient to cooperate with study visits and procedures.
11. Use of any of the restricted list of products known to strongly inhibit CYP3A4 metabolism (Appendix 15.4, Table 8) within 5 days prior to Day 1 dosing; or to strongly induce CYP3A4 metabolism (Appendix 15.4, Table 9) within 28 days prior to Day 1 dosing; or to strongly inhibit P-gp transporter (Appendix 15.4, Table 10) within 5 days prior to Day 1 dosing; or digoxin within 5 days prior to Day 1 dosing. For patients who require chronic inhaled glucocorticoid therapy, Investigators should confer with the Medical Monitor for additional guidance.
12. Serum bilirubin > ULN attributable to factors other than hemolysis and/or Gilbert's syndrome.
13. Male patients with heart-rate corrected QT (Fridericia's correction factor) QTcF interval > 450 msec, or female patients with QTcF interval > 470 msec with the exception of patients with a left bundle branch block (LBBB). Medical Monitor approval needed in patients with a LBBB.
14. Cardiac dysrhythmias judged as clinically significant by the Investigator or requiring therapy with drugs that are primarily substrates of CYP3A4.

15. History of allergy to sulfonamides if characterized by acute hemolytic anemia, anaphylaxis, or rash of erythema multiforme type or Stevens-Johnson syndrome.
16. Any other medical or psychological condition deemed by the Investigator to be likely to interfere with a patient's ability to understand and sign informed consent; cooperate with study visits, tests, and procedures; or otherwise safely and reliably participate in the study.

## 8.4. Patient Identification and Registration

Patients who are candidates for enrollment into the study will be evaluated for eligibility by the Investigator to ensure that the inclusion and exclusion criteria (see Section 8.2 and Section 8.3, respectively) have been satisfied and that the patient is eligible for participation in this clinical study. The site will submit to the Sponsor an Eligibility form for each eligible patient and the Medical Monitor will confirm eligibility for all patients prior to receipt of the first dose of AG-348.

## 8.5. Patient Randomization

Patients who have been confirmed as eligible will be randomized in an equal ratio to a treatment arm (e.g., 1:1 or 1:1:1 depending on which arms are open). The site will provide a request for randomization form (including the patient's confirmed genotype) to the study Medical Monitor. The randomization will be stratified by *PKR* mutation in order to maintain balance across the dose arms for the specific mutations expected to be most frequently enrolled. The *PKR* mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H) and all other mutations ("other"). Since this is an open label study, randomization will not be blinded.

Please refer to the study manual for the randomization procedure.

## 8.6. Patient Withdrawal Criteria

Patients have the right to withdraw from the study at any time for any reason. Patients will be withdrawn from study-related procedures and treatments under the following conditions:

- Withdrawal of consent;
- Experiences unacceptable toxicity;
- Development of an intercurrent medical condition that precludes further participation in the trial;
- Patient requires use of a prohibited concomitant medication (Section 9.11.2);
- Investigator decision;
- Protocol violation: non-adherence to protocol requirements;
- Pregnancy;
- Lost to follow-up.

Should a patient decide to withdraw, all efforts will be made to complete and report the protocol-defined study observations up to the time of the patient's withdrawal as completely as possible and to determine the reason for withdrawal.

In the event a patient is withdrawn from the study, the Medical Monitor must be informed. If there is a medical reason for withdrawal, the patient will remain under the supervision of the Investigator until satisfactory health is returned.

When a patient withdraws from the study, the primary reason for discontinuation must be recorded in the appropriate section of the electronic case report form (eCRF) and all efforts will be made to complete and report final study observations as thoroughly as possible.

All AEs should be followed until resolution or for a period of 30 days from the last dose of study drug, whichever is shorter. Patients with toxicity suspected to be related to study drug will continue follow-up until the AE resolves, is declared chronic by the Investigator, or the patient is lost to follow-up.

## 8.7. Replacement of Patients

Patients who drop out of the study prior to completing the first 12 weeks of assigned dosing for reasons other than AEs may be replaced at the Sponsor's discretion.

# 9. STUDY TREATMENT

## 9.1. Description of Study Drug

AG-348 sulfate hydrate capsules will be provided as 5 mg, 25 mg, or 100 mg (free-base equivalent) of AG-348 sulfate hydrate without excipients in dark green opaque (5 mg), Swedish orange (25 mg), or white opaque (100 mg) gelatin capsules (size 2 capsules for all dose strengths).

All study drugs are for investigational use only and are to be used only within the context of this study. All study drug products will be supplied by the Sponsor. Please see the Investigator's Brochure for further details regarding study drug.

## 9.2. Study Drug Packaging and Labeling

AG-348 sulfate hydrate capsules are packaged in white, high-density polyethylene (HDPE) induction sealed bottles with a child-resistant screw cap.

Packaging and labeling will be prepared to meet all regulatory requirements.

## 9.3. Study Drug Storage

AG-348 sulfate hydrate drug capsules must be stored at room temperature of 15 to 30°C (59 - 86°F).

All study drug products must be stored in a secure, limited-access location and may be dispensed only by the Investigator or by a member of the staff specifically authorized by the Investigator.

## 9.4. Method of Assigning Patients to Treatment

Up to a maximum of 25 patients will be randomized to any one of the dosing arms in this study. Randomization will be balanced 1:1 or 1:1:1, depending on whether 2 or potentially 3 arms are open, and will be stratified by *PKR* mutation (see Section 8.5). The dose and schedule of AG-348 each patient receives will be dependent upon which dose arm is open for enrollment when the patient qualifies for and is randomized into the study.

## 9.5. Blinding

This is an open-label study; no blinding methods will be used.

## 9.6. Study Drug Preparation and Administration

For the initial two treatment arms, (Arm 1 and Arm 2), AG-348 will be administered orally BID (approximately every 12 hours with a minimum of 10 hours between doses) over a 24 week treatment period. Starting with Day 1, dosing will be continuous; there will be no rest periods. Patients who do not meet any of the treatment withdrawal criteria (see Section 8.5) may continue treatment for the entire 24-week treatment period.

Patients will be dispensed the appropriate number of Sponsor-packaged, labeled bottles to allow for 28 days of dosing until the next scheduled visit.

Patients will be given a dosing diary to be used for each 28-day dosing period. They should record relevant information regarding their study drug in the diary (e.g., confirmation that each daily dose was taken, reasons for missed doses).

Treatment compliance will be assessed based on return of unused drug and the dosing diary (see Section 9.9).

Patients should be instructed to take their daily dose at approximately the same times each day except for dosing on in-clinic visiting days.

Patients who undergo extensive PK/PD sampling (see Appendix 15.1, Table 6) should be instructed from Week 3 on to bring the AM dose with them for in-clinic visits and to ingest the dose following PK/PD blood draws.

Patients receiving limited PK/PD sampling (see Appendix 15.1, Table 7) should be instructed to bring the AM dose with them for all in-clinic visits and to take the AM dose following PK/PD blood draws.

Patients receiving extensive PK/PD sampling on Day 1 and 15 will also have limited PKPD on other visit days. As a general rule, regardless of extensive or limited schedule, patients will bring in the AM dose for all visits and take this dose following PK/PD blood draws.

A minimum of 10 hours between the AM and PM dose will be required on those dosing days. Each dose should be taken with a glass of water and consumed over as short a time as possible. AG-348 may be taken with or without food. Patients should be instructed to swallow capsules whole and to not chew the capsules. For patients who have difficulty swallowing tablet(s), the Medical Monitor should be contacted to discuss administration.

Patients will receive their first dose of AG -348 in the clinic on Day 1 and then may take the remaining doses on an outpatient basis.

## 9.7. Criteria for Dose Escalation, Dose Modification, or Discontinuation of Study Drug

No intra-patient dose escalations will be permitted in this study unless the DRT decides to re-assign patients' doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the patients in the terminated arm will remain in their originally assigned arm; i.e., they will not count towards the enrollment quota of the arm whose dose and schedule is being adopted. All dosing modifications, as outlined below, will be implemented following discussions with the Medical Monitor.

### 9.7.1. Dose Modification for Safety

The Investigator will monitor all patients for safety and tolerability. Modification of the patient's dose of AG-348 will be based on AEs and observed changes in Hb levels (see Section 9.7.2).

**Table 1: Dose Modification for Adverse Events Deemed at Least Possibly Related to AG-348**

| Adverse Events(s) | AG-348 Dose Adjustment |
|---|---|
| Grade 1 | None required. |
| Grade 2 | None required; Investigator and Medical Monitor judgment to manage as for Grade 3. |
| Grade 3 | Suspend dosing;<br>If event resolves to Grade 1 or baseline within approximately 14 days of suspension, resume dosing with 1 dose level reduction (see Table 2 below).<br>If event does not resolve to Grade 1 or baseline within approximately 14 days of suspension, permanently discontinue dosing, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor. |
| Grade 4 | Permanently discontinue dosing, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor. |

Dose modifications for Grade 2 and 3 AEs should be maintained long enough for the Investigator to be confident that the reduced dose is being well tolerated before considering a request of the Medical Monitor to permit subsequent re-escalation to the former dose level. Dosing for an individual patient will be discontinued permanently for Grade 3 and Grade 4 AEs that do not resolve to Grade 1 or baseline within approximately 14 days of suspension of dosing, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor. No patients may be re-escalated to their former dose level after a dose modification without discussion with the Medical Monitor. If following the first dose reduction for a Grade 3 AE the patient experiences a second occurrence of the same Grade 3 AE, then treatment with AG-348 must be immediately and permanently discontinued.

It should be noted that if the initial dose of 300 mg BID selected for Arm 1 demonstrates an unacceptable safety profile resulting in multiple subjects undergoing dose modifications, the DRT may exercise its option to re-assign these patients' dose and schedule to match the dose and schedule of another study arm (for example, Arm 2 of the study, or to match the dose and schedule of a [potential] Arm 3, if implemented).

### 9.7.2. Dose Modification for Increase in Hemoglobin Level

It is presently unknown to what magnitude, how rapidly, or even whether, AG-348 will result in increased Hb levels in patients with PK deficiency across the variety of potential *PKR* mutations that may be encountered during this study. Moreover, it is unknown whether any potential increases in Hb experienced by patients in this study may be a safety risk. As a conservative measure, this study incorporates guidance for dose modification based on potential large increases in Hb levels that exceed the midpoint of the typical normal range by gender. The Investigator will monitor all patients for changes in Hb levels and should adjust the dose of AG-348 as outlined in Table 2 per the following guidelines and with Medical Monitor approval.

- Males: If Hb > 15 g/dL and confirmed with a second test, suspend dosing until Hgb ≤ 13.5 g/dL; then resume dosing with a 1 dose level reduction.

- Females: If Hb > 13.5 g/dL and confirmed with a second test, suspend dosing until Hb ≤ 12.5 g/dL; then resume with a 1 dose level reduction.
- The treating Investigator will discuss with the Medical Monitor questions relating to dose modifications on an as needed basis.

**Table 2: Dose Reduction Table (by Dosing Arm)**

| Dose Group | Starting Dose | 1st Dose Reduction | 2nd Dose Reduction |
|---|---|---|---|
| Arm 1 | 300 mg BID | 200 mg BID | 100 mg BID |
| Arm 2 | 50 mg BID | 25 mg BID | TBD[1] |
| Potential Arm 3 | TBD | To approximately 50-66% of initial dose | To approximately 25-33% of initial dose |

[1] Dose to be determined by Medical Monitor.

Hemoglobin levels above the ULN by gender should be reported as an AE, graded per the CTCAEv4.03, according to the guidance provided in Section 11.2.

### 9.7.3. Stopping Criteria

Dosing for an individual patient will be discontinued permanently for Grade 3 AEs that do not resolve to Grade 1 or baseline within approximately 14 days of suspension of dosing, and for Grade 4 AEs, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor (Section 9.7.1). Other reasons for treatment termination are provided in Section 8.5.

## 9.8. Duration of Patient Participation

The duration of treatment for all patients on this study will be up to 24 weeks. Patients who safely tolerate and demonstrate one or more indicators of clinical activity of AG-348 may be eligible to immediately roll over to a safety extension for continued treatment.

## 9.9. Treatment Compliance

During in-clinic visits, doses of AG-348 will be ingested by the patient under the supervision of clinical facility personnel. For at-home dosing, patients will be given a dosing diary to be used for the duration of the 24-week treatment period. Patients should record relevant information regarding their study drug in the diary (e.g., confirmation that each daily dose was taken, reasons for missed doses) and return the diary at each study visit.

## 9.10. Study Drug Accountability

Accountability for the study drug at the clinical facility is the responsibility of the Investigator. The Investigator will ensure that the study drug is used only in accordance with this protocol. Where allowed, the Investigator may choose to assign drug accountability responsibilities to a pharmacist or other appropriate individual.

The Investigator or delegate will maintain accurate drug accountability records indicating the drug's delivery date to the site, inventory at the site, use by each patient, and return to Agios or its designee (or disposal of the drug, if approved by Agios). These records will adequately

document that the patients were provided the doses as specified in the protocol and should reconcile all study drug received from Agios. Accountability records will include dates, quantities, batch/serial numbers, expiration dates (if applicable), and patient numbers. An unblinded monitor will review drug accountability at the site on a schedule agreed to by the Sponsor.

Study drug must not be used for any purpose other than the present study.

All unused and used study drug will be retained at the site until it is inventoried by the Study Monitor after database lock has occurred. All used, unused or expired study drug will be returned to Agios or its designee or, if authorized, disposed of at the study site per the site's Standard Operating Procedures (SOPs) and documented. All material containing AG-348 will be treated and disposed of as hazardous waste in accordance with governing regulations.

## 9.11. Prior and Concomitant Medications and Treatments

### 9.11.1. Prior Medications and Procedures

All medications administered and procedures conducted within 28 days prior to the first day of study drug administration are to be recorded on the source documentation and included in the eCRF.

### 9.11.2. Prohibited Concomitant Therapy

All concomitant medications and procedures administered from 28 days before administration of study drug through the last Follow-up Visit (Week 28/Day 197) must be recorded in the appropriate section of the source documentation and eCRF along with dosage information, dates of administration, and reason for use.

The following are prohibited at all times during participation in this study:

- Investigational drugs must be discontinued 28 days prior to the first dose of study drug;
- Products known to strongly inhibit CYP3A4 metabolism (listed in Appendix 15.4, Table 8) must be discontinued within 5 days prior to Day 1 dosing;
- Products known to strongly induce CYP3A4 metabolism (listed in Appendix 15.4, Table 9) must be discontinued within 28 days prior to Day 1 dosing;
- Products known to strongly inhibit P-gp transporter (listed in Appendix 15.4, Table 10) must be discontinued within 5 days prior to Day 1 dosing;
- Digoxin must be discontinued within 5 days prior to Day 1 dosing;
- Hematopoietic stimulating agents (erythropoietins, granulocyte colony stimulating factors, thrombopoietins, etc) must be discontinued no less than 28 days prior to the first dose of study drug. [Folic acid 1 mg orally per day is required for all patients. B12 injections are permitted for patients with a prior diagnosis of B12 deficiency syndromes, but must be repleted to stability of the Hb and mean corpuscular volume (MCV) prior to enrollment in the study];

- Anabolic steroids, including testosterone preparations, administered for anemia must be discontinued no less than 28 days prior to the first dose of study drug;
- As the target population for this study consists of transfusion independent patients and transfusion of blood products could confound key endpoints of the study, blood transfusions of any type must be strictly avoided except in cases of compelling medical need. If medical circumstances permit, the Medical Monitor should be contacted for discussion before any transfusions are administered.

Drugs that displace unconjugated bilirubin from albumin (including some common sulfa antibiotics [including sulfamethoxazole/trimethoprim], cephalosporins, salicylates, and aminophylline) should be used with caution with the understanding that patients with elevated levels of unconjugated bilirubin may potentially be at risk for kernicterus syndrome (Strauss, et al. 2006).

**Potential Drug Interactions**

Strong inhibitors of CYP3A4 (listed in Appendix 15.4, Table 8) are not permitted for use with AG-348. Based on modeling of AG-348 metabolism and tolerability data from the Phase 1 MAD study, moderate inhibitors of CYP3A4 do not appear to pose a risk to patients dosed with AG-348.

In-vivo data from the Phase 1 MAD show AG-348 induces CYP3A4 and its own metabolism, presumably via CYP3A4. The use of strong CYP3A4 inducers in combination with AG-348 is expected to reduce AG-348's efficacy. Therefore, administration of strong CYP3A4 inducers (listed in Appendix 15.4, Table 9) is not permitted with AG-348.

Strong inhibitors of drug transport (listed in Appendix 15.4, Table 10) are not permitted for use with AG-348.

Digoxin is not permitted for use with AG-348.

Induction of CYP3A4 by AG-348 is expected to reduce the efficacy of certain sensitive co-medications. Co-administration of AG-348 with the drugs in Appendix 15.4, Table 11 should be done with caution, as their efficacy may be reduced.

Of note, women in the trial utilizing oral contraception must utilize barrier methods as per the Inclusion Criteria 14 (Section 8.2) while taking AG-348.

Short-term (≤ 14 days at a time, and ≤ 28 days total during the 24 week treatment period) use of topical, inhaled, intra-nasal, and systemic glucocorticoids is permitted for acute medical indications. Every effort should be made to minimize total duration of glucocorticoid therapy and utilize alternative treatments. Patients must be off glucocorticoids for at least 28 days prior to Day 1 of AG-348 dosing as per Exclusion Criterion #11 (Section 8.3). For patients who require chronic inhaled glucocorticoid therapy, Investigators should confer with the Medical Monitor for additional guidance.

The expected patient co-medications deferoxamine, deferasirox, deferiprone, and oral penicillin are not expected to interact with AG-348.

### 9.11.3. Allowed Concomitant Therapy

Medications and treatments other than those specified above are permitted during the study. All intercurrent medical conditions will be treated at the discretion of the Investigator according to acceptable local standards of medical care. Subjects may receive analgesics, antiemetics, anti-infectives (including penicillins), and antipyretics as medically indicated and consistent with the guidance in the previous two sections. Patients may continue iron chelation therapy with deferoxamine and deferasirox. Patients must continue taking 1 mg of folic acid for the duration of the study.

All concomitant medications, including transfusions of blood products, procedures performed during the study, including those used to treat AEs, will be recorded on the eCRF.

### 9.11.4. Potential for Phototoxicity

AG-348 may cause sensitivity to direct and indirect sunlight. Patients should be warned to avoid direct sun exposure. When exposure to sunlight is anticipated for longer than 15 minutes, the patient should be instructed to apply factor 30 or higher sunscreen to exposed areas and wear protective clothing and sunglasses.

### 9.11.5. Management of Nausea, Vomiting, and Diarrhea

As the primary objective of this study is to assess the safety and tolerability of up to 24 weeks of AG-348, routine use of prophylactic anti-emetic and antidiarrheal or other classes of medications is prohibited. However, if patients experience AEs relating to nausea, vomiting, or diarrhea these may be treated at the Investigator's clinical discretion with recommended medications as follows:

- Nausea/vomiting: allowed agents include standard clinical dosing with palonosetron (Aloxi), dolasetron (Anzemet), or promethazine (Phenergen). Promethazine is a substrate for CYP2B6, and it is presently unknown if the potential for 2B6 induction after AG-348 dosing could be sufficient to reduce the therapeutic effect of promethazine. Not recommended are aprepitant (Emend; CYP3A4 inhibitor), ondansetron (Zofran; CYP3A4 inducer), chlorpromazine (Thorazine; CYP3A4 inducer), prochlorperazine (Compazine; CYP3A4 substrate, QT prolongation has been reported), and granisetron (Kytril; CYP3A4 substrate, QT prolongation has been reported);
- Diarrhea: recommended management includes standard clinical dosing with Kaopectate or other non-absorbable anti-diarrheals, diphenoxylate/atropine (Lomotil), or loperamide (Imodium). Loperamide is the least preferred choice because it is both a substrate and inhibitor for CYP3A4, a substrate for CYP2B6, and a substrate for P-gp.
- For the use of any medications not specifically mentioned above the Investigator may confer with the Sponsor’s Medical Monitor.

### 9.11.6. Other Restrictions and Precautions

Patients should be advised to refrain from altering their normal exercise routine for the first 28 days of treatment with AG-348 as symptoms of anemia could theoretically temporarily worsen before the eventual potential beneficial effect of AG-348 on the Hb level becomes

evident. The elevated blood levels of 2,3-DPG commonly seen in patients with PK deficiency may produce a right shift in the Hb-O2 dissociation curve. The effect of this is to enhance oxygen delivery at the tissue level, thus counteracting to some extent the physiologic consequences of the anemia. AG-348 was shown to produce rapid decreases (within the first 12 hours following a single dose) in 2,3-DPG in two prior clinical trials with healthy adult male and female volunteers. In patients with PK deficiency who have elevated 2,3-DPG levels, it is theoretically possible that decreases in 2,3-DPG that precede correction of the anemia could result in a temporary decrease of oxygen delivery at the tissue level resulting in clinical symptoms (e.g., increased fatigue).

As discussed in Section 5.2.1.1 of this protocol and in the Investigator Brochure, AG-348 has been identified as a histamine H3 receptor antagonist/inverse agonist. No effects of histamine H3 modulation have been observed in safety pharmacology or toxicology studies. Nonetheless, patients should be monitored for potential adverse events related to wakefulness and insomnia (Schwartz 2011).

# 10. STUDY ASSESSMENTS

## 10.1. Schedule of Assessments

The Schedules of Assessments for this study are provided in Appendix 15.1.

After obtaining written informed consent, patients will undergo screening evaluations. The Screening visit is to be conducted within approximately 28 days prior to first dose of study treatment.

During the Treatment period, patients will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12) and monthly through Week 24 (Weeks 16, 20, and 24). Patients who safely tolerate and demonstrate one or more indicators of clinical activity of AG-348 through Week 24 may be eligible to immediately enter a safety extension for continued treatment upon agreement of the treating Investigator and the Medical Monitor. For patients who finish treatment, Study Discharge will occur 4 weeks (Week 28 or earlier) following the last dose of study treatment at the final follow-up assessment.

Although *not* encouraged, as a convenience for patients who travel long distances to the study site, in-clinic visits on Day 8 and Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis. For details, please refer to Table 5 in Appendix 15.1 : Schedule of Assessments. For patients having their Day 8 and/or Day 22 visits performed by the primary care physician, the principal investigator will him or herself, or have a qualified research nurse or other designated site staff member, make telephone contact with the patient to inquire about any adverse events. These must be recorded as if the patient appeared in the main study center. The Principal Investigator will exercise prudent clinical judgment in determining any clinical course of action to take based on any AEs discovered. These telephone contacts on Day 8 and Day 22 must be explained to the patient in advance and scheduled in advance to maximize the likelihood of successfully making contact. Having in-clinic visits on Days 8 and/or Day 22 performed by the primary care physician will necessitate re-scheduling certain assessments that the primary care physician's office may not be reasonably expected to perform. Table 3 summarizes the details of the re-scheduling of these assessments as described in Table 5 in Appendix 15.1.

**Table 3: Summary of Assessments When Day 8 and/or Day 22 In-Clinic Visits Are Performed By Primary Care Physician**

| Day 8 Visit by Primary Care Physician | | | | Day 22 Visit by Primary Care Physician | | | |
|---|---|---|---|---|---|---|---|
| Primary Care Office | Main Study Site | Assessment to Move to Day 15 Main Study Site | Assessments Not Required | Primary Care Office | Main Study Site | Assessment to Move to Day 43 Main Study Site | Assessments Not Required |
| Hematology to central lab | Phone contact with patient | 12-lead ECG | VS; serum chemistry | Hematology sample to central lab | Phone contact with patient | 12-lead ECG | VS; serum chemistry; coagulation, haptoglobin, EPO level, carboxyhemoglobin, PK/PD |

Abbreviations: ECG = electrocardiogram; EPO = erythropoietin; PK/PD = pharmacokinetics/pharmacodynamics; VS = vital signs

Whenever more than one assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (e.g., VS first, ECG, blood draw). The timing of these assessments should allow the PK blood draw to occur at the exact nominal time. The order of procedures may be revised with prior discussion between Sponsor and Site.

Minor adjustments to the timing, number of planned safety monitoring procedures (e.g., VS, ECG, blood draw), and PK/PD assessments may be made during the course of the study based on collected data to ensure appropriate safety monitoring and will not require a protocol amendment. These minor changes will require prior approval from the Sponsor's Medical Monitor (or Responsible Medical Officer) as well as appropriate documentation in the study records. The addition of new safety monitoring procedures or other assessments will require a protocol amendment.

## 10.2. Informed Consent and Confirmation of Eligibility

A complete description of the study is to be presented to each potential patient and a signed and dated informed consent is to be obtained before any study specific procedures are performed.

Patient's eligibility will be confirmed at Screening and within 24 hours prior to study treatment dose. If a patient is determined to be ineligible, the patient will be excluded from participation.

## 10.3. Demographic Data, Medical and Medication History

Patient demographic data, including gender, date of birth, age, race, and ethnicity, will be obtained at Screening. Collection of demographic data will be modified by country regulatory requirements, as appropriate.

Medication history, including all relevant prior medical history and current medical conditions, will be obtained at the Screening assessment and on Day -1; all concomitant medications administered and procedures conducted within 28 days prior to Day 1 should be reported in the source documentation and eCRF. Investigators will be asked to provide information on the patient's history of any medical diagnoses (e.g., iron overload) and surgical procedures (e.g., splenectomy, cholecystectomy) pertaining to their diagnosis of PK deficiency and prior available

complete blood counts (CBCs) over the preceding 6 months and transfusion history over the preceding 12 months prior to the date of signing informed consent.

## 10.4. PKR Enzymatic Assay and PKR Genotyping

Assessments for PKR enzymatic activity and PKR genotyping will be performed at Screening only for confirmation of study eligibility. PKR enzymatic assays will be conducted at [redacted]. PKR genotyping will be conducted at [redacted]

## 10.5. Safety Assessments

### 10.5.1. Physical Examination, Height, and Weight

A complete physical examination (including neurological examination; genital and rectal examinations will be performed at the discretion of the Investigator) will be obtained at Screening, Baseline, Week 12, and the Follow up Visit (Week 28). The neurological examination must include an assessment of general orientation and mental status including level of alertness (assess as normal or abnormal and specify any abnormality). For patients rolling over to the safety extension, the last physical examination will occur at Week 24. Limited focused physical examinations will be performed at all other visits. Any findings will be recorded on the eCRF. Height will be collected at Screening only.

### 10.5.2. Vital Signs

Vital signs, including systolic and diastolic BP, heart rate, respiratory rate, and temperature, will be obtained according to the according to the Schedule of Assessments (Appendix 15.1). Vital sign measurements will be repeated if clinically significant or machine/equipment errors occur. Out of range BP or heart rate measurements will be repeated at the Investigator's discretion. Any confirmed, clinically significant vital sign measurements will be recorded as AEs.

### 10.5.3. Electrocardiogram

A 12-lead ECG will be obtained according to the Schedule of Assessments (Appendix 15.1). The ECGs will be measured using an ECG machine that calculates the heart rate and measures PR, QRS, QT, QTcB (Bazett correction formula), and QTcF (Frederica's correction). Only QTcF (not QTcB) will be used for determination of eligibility.

The 12-lead ECGs should be obtained following 5 minutes of recumbency. The Screening ECG will be performed at least 7 days prior to Day 1 dosing. ECGs will be repeated if clinically significant abnormalities are observed, if artifacts are present, or if machine/equipment errors occur. Any confirmed, clinically significant ECG findings will be recorded as AEs.

### 10.5.4. DXA Scans

DXA scans (hip and spine) will be performed at Screening to obtain T and Z scores that will serve as a baseline measure of bone mineral density for all enrolling patients. A second DXA scan will be conducted in the interval between Week 24 and Week 28 as indicated in the Schedule of Assessments (Appendix 15.1). All redacted DXA scan reports must be held at the study site and will be collected by the Sponsor.

### 10.5.5. Safety Laboratory Assessments

#### 10.5.5.1. Hematology, Serum Chemistry, Coagulation Studies, and Urinalysis

Laboratory values obtained prior to Screening and RBC antibodies obtained at Screening will be performed at a local laboratory. On-study clinical laboratory evaluations are to be performed by a central laboratory. If Investigators believe that it is clinically indicated to obtain safety laboratory results from their own local laboratories on the day of the subject's visit, and before the results are returned from the designated central laboratory, they are free to exercise their discretion to do so. Investigators should be aware that since the Hb level is a secondary endpoint of the study, it behooves them to minimize blood volumes drawn, and samples for the central lab must still be collected, since it will serve as the official lab result for this study.

Clinical laboratory evaluations are to be collected according to the Schedule of Assessments (Appendix 15.1). In addition, all clinically significant laboratory abnormalities noted on testing will be followed by repeat testing and further investigated according to the judgment of the Investigator. Please note that serum estradiol, free and total testosterone, and complete blood count (CBC) will be collected in the AM at any 2 time points during Screening at least 2 days apart in addition to Baseline/Day 1 (total of 3 time points prior to Day 1 dosing).

The following safety laboratory parameters are to be determined:

| | |
|---|---|
| **Hematology**: | HCT, Hb, RBC count, absolute reticulocyte count, percent reticulocyte count, MCV, mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count with automated (or manual, as indicated) differential, ANC, and absolute lymphocyte count (ALC), and platelet count. In the event that the designated central laboratory for the study is unable to provide a valid result for any specific component of the defined CBC for a specific patient, the site may be asked to have the test performed at their local laboratory. The result of the local CBC will be entered into the study database along with the local normal reference range. G6PD and RBC antibody screen will be performed at Screening only |
| **Other** | EPO, Hp, COHb, 25-hydroxy vitamin D2 and D3. |
| **Serum Chemistry:** | alkaline phosphatase (ALP), sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide ($CO_2$) or bicarbonate, albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, LDH, ALT, AST, total bilirubin, indirect bilirubin, (and estimated creatinine clearance or glomerular filtration rate for screening only, as appropriate). |
| **Sex Hormones**: | testosterone (total and free), estrone, and estradiol. FSH will only be performed at Screening for female patients only for confirmation of post-menopausal status. |

| | |
|---|---|
| **Bone Turnover** | serum osteocalcin-N-mid and CTX. |
| **Lipids** | total cholesterol, HDL-C, triglycerides. |
| **Iron Panel** | iron (Fe), total iron-binding capacity (TIBC), transferrin saturation, ferritin. |
| **Coagulation Studies:** | fibrinogen, aPTT, INR. |
| **Urinalysis**: | color and appearance, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, pregnancy screen (dipstick), occult blood. Microscopic inspection of sediment will only be performed for cause or to investigate an abnormal dipstick finding per the Investigator's discretion. |

#### 10.5.5.2. Screening Serology

A blood sample for serology, including HBsAg, HCV Ab screen, and HIV1 and HIV2 Ab, is to be collected from all patients at Screening.

### 10.5.6. Menstrual Cycle Diary

Menstruating female patients will be required to fill out a paper-based menstrual cycle diary each month in order to monitor any changes. Diaries will be dispensed and collected as indicated in the Schedule of Assessments (Appendix 15.1). Patients will record the start date, stop date, and any notable characteristics of each menstrual cycle.

### 10.5.7. Adverse Events

Each patient will be carefully monitored for the development of any AEs throughout the study from signing of the informed consent through all scheduled study follow-up visits, or withdrawal of consent, whichever occurs first. In addition, SAEs that are assessed as possibly or probably related to study treatment that occur > 30 days post-treatment also are to be reported.

AEs will be evaluated by the Investigator and recorded as described in the Schedule of Assessments. On dosing visits, all AEs (elicited and spontaneously reported) will be continuously evaluated by the Investigator and recorded. At any non-dosing day visit, AEs will be evaluated by the Investigator and recorded. Any AEs already documented at a previous assessment and designated as ongoing will be reviewed at subsequent visits or assessment time points as necessary. If these AEs have resolved, this will be documented.

All AEs will be graded using the CTCAE grading system (Appendix 15.2).

Complete details on AE monitoring are provided in Section 11.

## 10.6. Pharmacokinetic Assessments

### 10.6.1. Blood Sample Collection and Pharmacokinetic Measurements During Dose Escalation

The first approximately 10 patients treated, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 15.1, Table 6. The remainder of treated patients will undergo limited PK sampling as detailed in Appendix 15.1, Table 7. The in-clinic visit on Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis. In this instance, PK sampling will not be required on Day 22. (Additional details regarding Day 8 and Day 22 visits performed by the patient's primary care physician can be found in Table 5 in Appendix 15.1: Schedule of Assessments.)

The collection times for post-dose PK samples will start from the time that dosing is completed. (For example, a PK draw at 30 minutes will be collected 30 minutes after the last capsule has been ingested.) The completion time of each dose will be recorded.

Procedures for sample collection and processing will be provided in a separate study manual. The actual time point of each sample collection will be recorded.

Samples for PK and PD assessments may be retained for up to 2 years from collection.

## 10.7. Pharmacodynamic Assessments

The first approximately 10 patients treated, contingent on clinical site feasibility, will undergo extensive PD sampling for 2,3-DPG and ATP as detailed in Appendix 15.1, Table 6. The remainder of treated patients will undergo limited PD for 2,3-DPG and ATP sampling as detailed in Appendix 15.4, Table 7.

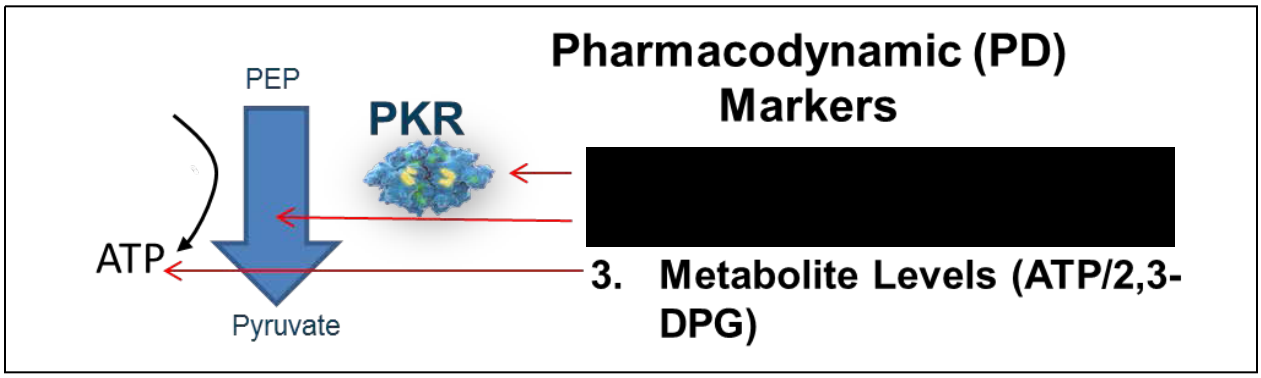
The collection times for post-dose PD samples will start from the time that dosing is completed. (For example, a PD draw at 30 minutes will be collected 30 minutes after the last capsule has been ingested.) The completion time of each dose will be recorded.

Procedures for sample collection and processing will be provided in a separate study manual. The actual time point of each sample collection will be recorded.

Pharmacodynamic assessments will include 2,3-DPG, ATP, [redacted] The in-clinic visit on Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis. In this instance, PD sampling will not be required on Day 22. (Additional details regarding Day 8 and Day 22 visits performed by the patient's primary care physician are stated in Table 5 in Appendix 15.1: Schedule of Assessments.)

Figure 4 provides a brief schematic outlining the PKR reaction and how each of these PD assessments fits into a complete mechanistic understanding of the action of AG-348.

**Figure 4: PKR Enzymatic Reaction**




The PKR enzyme catalyzes the PEP to pyruvate reaction, with concomitant formation of ATP.

- Binding of AG-348 to the PKR tetramer can be assessed through an ex-vivo biochemical assay of cell lysates from AG-348 treated patients. Because WBCs contain a high level of pyruvate kinase from a non-PKR pyruvate kinase isoform, WBCs are first removed by filtration before the purified red cells are frozen. [redacted]
- [redacted]
- AG-348 target engagement [redacted] has been shown in preclinical models and healthy volunteer clinical studies to result in accumulation of ATP and depletion of the upstream metabolite 2,3-DPG. Therefore, levels of these metabolites will be measured by mass spectrometry in frozen whole blood samples.

The first approximately 10 patients treated, contingent on clinical site feasibility, will undergo extensive PD sampling as detailed in Appendix 15.1, Table 6. The remainder of treated patients will undergo limited PD sampling as detailed in Appendix 15.1, Table 7. The collection times for post-dose PD samples will start from the time that dosing is completed. (For example, a PD draw at 30 minutes will be collected 30 minutes after the last capsule has been ingested.) The completion time of each dose will be recorded.

Blood samples will be stored at the site and regularly transported at -80°C ±10 C to the bioanalytical laboratory for analysis. Procedures for sample collection and processing will be provided in a separate study manual.

## 10.8. Ordering of Blood Sample Collection

When more than 1 blood sample is collected at the same nominal time, the samples will be collected in the following order:

1. Safety laboratory assessments
2. PK
3. PD (2,3 DPG, ATP)

[redacted]

## 10.9. Sample Processing, Storage, and Shipment

Instructions for the processing, storage and shipment of all study samples for central analysis will be provided in a separate study manual.

# 11. ADVERSE EVENTS

Monitoring of AEs will be conducted throughout the study. Adverse events and SAEs will be recorded in the source documentation and eCRF from time of the signing informed consent through the Follow-up Visits for randomized patients. All AEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

All SAEs will be followed through 30 days after the last dose of study treatment or until the SAE has resolved. Any SAEs that are assessed as possibly or probably related to study treatment that occur > 30 days post-treatment also are to be reported.

## 11.1. Definition of Adverse Events

### 11.1.1. Adverse Event (AE)

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An AE can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

### 11.1.2. Suspected Adverse Reaction

A suspected adverse reaction is any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of expedited safety reporting, 'reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the AE.

### 11.1.3. Unexpected Adverse Event

An unexpected AE is one for which the nature or severity of the event is not consistent with the applicable product information, e.g., the Investigator's Brochure.

### 11.1.4. Serious Adverse Event

An AE or suspected adverse reaction is considered serious (SAE) if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death;
- Life-threatening. Life-threatening means that the patient was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form;
- In-patient hospitalization or prolongation of existing hospitalization. Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered AEs if the illness or disease existed before the patient was enrolled in the study, provided that it did not deteriorate in an unexpected manner during the study (e.g., surgery performed earlier than planned);

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- Congenital anomaly/birth defect;
- Important medical event. An important medical event is an event that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the patient or patient and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions for SAEs. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalization, or the development of drug dependency or drug abuse.

#### 11.1.4.1. Potential Severe Drug-Induced Liver Injury

The document entitled FDA Guidance for Industry, Drug-Induced Liver Injury: Premarketing Clinical Evaluation (FDA 2009) provides guidance on how the measurement of various laboratory parameters may be used to assess a given drug's potential to cause severe liver injury (i.e., irreversible liver failure that is fatal or requires liver transplantation). Such cases are suggested by the following three components:

1. The drug causes hepatocellular injury, generally shown by a higher incidence of 3-fold or greater elevations above the ULN of ALT or AST than the (non-hepatotoxic) control drug or placebo;
2. Among trial patients showing such aminotransferase elevations, often with aminotransferases much greater than 3×ULN, one or more also show elevation of serum total bilirubin to > 2×ULN, without initial findings of cholestasis (elevated serum elevated serum ALP);
3. No other reason can be found to explain the combination of increased aminotransferases and total bilirubin, such as viral hepatitis A, B, or C; pre-existing acute liver disease; or another drug capable of causing the observed injury.

Clinical safety laboratory results compatible with the definition of drug-induced liver injury (DILI) stated above must be repeated for confirmation as soon as possible, and if confirmed, will be scored as an unacceptable AE and reported to FDA as a serious unexpected AE.

## 11.2. Procedures for Reporting Adverse Events and Serious Adverse Events

Each patient must be carefully monitored for the development of any AEs. This information should be obtained in the form of non-leading questions (e.g., "How are you feeling?") and from signs and symptoms detected during each examination, observations of study personnel, and spontaneous reports from patients.

All AEs (serious and non-serious) spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures will be recorded in the source documentation and eCRF. Any clinically relevant deterioration in laboratory assessments or other clinical findings is considered an AE

and must be recorded in the appropriate source documentation and eCRF. Treatment-emergent abnormal clinical laboratory results should generally be reported as AEs if there are accompanying symptoms; if additional diagnostic evaluations or medical (including drug therapy) or surgical interventions are undertaken; if a change in study drug dosing or study drug discontinuation is required; or, if the laboratory result is considered clinically significant by the Investigator. Although it is an objective of this study to determine if treatment with AG-348 results in increased Hb levels in patients with PK deficiency, overshoot of the Hb level above the ULN by gender is not recommended and should be reported as an AE, graded per the CTCAEv4.03. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

All SAEs that occur during the course of the study must be promptly reported by the Investigator to [redacted] Global Safety and Pharmacovigilance (see below). Deaths and AEs assessed as life-threatening are to be reported immediately and SAEs that meet other criteria are to be reported within 24 hours from the point in time when the Investigator becomes aware of the SAE. All SAEs must be reported whether or not they are considered causally related to AG-348. Serious adverse event forms will be completed and the information collected will include subject number, a narrative description of the event, and an assessment by the Investigator as to the severity of the event and relatedness to study drug. Follow-up information on the SAE may be requested by the Sponsor or Medical Monitor.

**Contact Information:**

[redacted] **Safety Management**

SAE Hotline (United States): [redacted]
SAE Fax (United States): [redacted]

SAE Hotline (France): [redacted]
SAE Fax (France): [redacted]

SAE Hotline (Netherlands): [redacted]
SAE Fax (Netherlands): [redacted]

SAE Hotline (Italy): [redacted]
SAE Fax (Italy): [redacted]

SAE Hotline (United Kingdom): [redacted]
SAE Fax (United Kingdom): [redacted]

E-mail: [redacted]

If there are serious, unexpected adverse drug reactions associated with the use of AG-348, the Sponsor will notify the appropriate regulatory agency(ies) and all participating Investigators on an expedited basis. The local Institutional Review Board (IRB)/Independent Ethics Committee (IEC) will be promptly notified based on local regulations where required by the IRB/IEC of all serious, unexpected adverse drug reactions involving risk to human subjects.

All AEs, whether serious or not, will be described in the source documents in the database. All new events, as well as those that worsen in intensity or frequency relative to baseline, which occur after signing the informed consent through the final Follow-up Visit (Day 29 ± 3 days) must be recorded. Adverse events that are ongoing at the time of treatment discontinuation

should be followed up to 30 days after the last dose of study treatment. All SAEs will be followed up to 30 days after the last dose of study treatment, or until the SAE has resolved.

Information to be reported in the description of each AE includes:

- A medical diagnosis of the event (if a medical diagnosis cannot be determined, a description of each sign or symptom characterizing the event should be recorded);
- The date of onset of the event;
- The date of resolution of the event;
- Whether the event is serious or not;
- Intensity of the event (see below for definitions);
- Relationship of the event to study treatment (see below for definitions);
- Action taken: none; change in the study drug administration (e.g., temporary interruption in dosing); drug treatment required; non-drug treatment required; hospitalization or prolongation of hospitalization required (complete SAE page); diagnostic procedure performed; patient discontinued from the study (complete End of Study visit);
- Outcome: patient recovered without sequelae; patient recovered with sequelae; event ongoing; patient died (notify the Medical Monitor immediately, and complete the SAE form).

**Intensity** of all AEs will be graded according to the NCI CTCAE Version 4.03 (Appendix 15.1).

**Relationship** to study drug administration will be determined by the Investigator according to the following criteria:

- **Not Related**: Exposure to the study treatment did not occur, or the occurrence of the AE is not reasonably related in time, or the AE is considered unlikely to be related to the study treatment.
- **Possibly Related**: The study treatment and the AE were reasonably related in time, and the AE could be explained equally well by causes other than exposure to the study treatment. An attribution of possibly related means that there are facts in evidence to suggest a possible relationship.
- **Probably Related**: The study treatment and the AE were reasonably related in time, and the AE was more likely explained by exposure to the study treatment than by other causes, or the study treatment was the most likely cause of the AE. An attribution of probably related means that there are facts in evidence to suggest a probable relationship.

For the purpose of safety analyses related to final database review, all AEs that are classified as possible or probable will be considered treatment-related AEs.

## 11.3. Pregnancy Reporting

Pregnancy is neither an AE nor an SAE, unless a complication relating to the pregnancy occurs (e.g., spontaneous abortion, which may qualify as an SAE). However, any pregnancy in a participating female patient or a female partner of a participating male patient that occurs during this study or within 30 days following the last dose of AG-348 must be reported to the Sponsor or Medical Monitor within 30 days of being notified of the pregnancy. The Investigator must follow up and document the course and outcome of all pregnancies even if the patient was discontinued from the study or if the study has finished. The female patient or partner of a male patient should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus. Monitoring should continue until conclusion of the pregnancy.

All outcomes of pregnancy must be reported by the Investigator to the Sponsor or Sponsor's designee on a Pregnancy Outcome Report form within 30 days after he/she has gained knowledge of the delivery or elective abortion.

Any SAE that occurs during pregnancy must be recorded on the SAE report form (e.g., maternal serious complications, spontaneous or therapeutic abortion, ectopic pregnancy, stillbirth, neonatal death, congenital anomaly, or birth defect) and reported within 24 hours in accordance with the procedure for reporting SAEs.

All patients, male and female, must agree to use effective contraception during the entire study and for 30 days following the last dose of AG-348. Abstinence is an acceptable method only when this is in line with the normal life style of the patient, meaning that the patient plans to remain abstinent *continuously* throughout the duration of the study and for at least 30 days after the last dose of study drug. Periodic abstinence, e.g. calendar, sympathothermal and post-ovulation methods, and withdrawal are not acceptable methods of contraception.

# 12. STATISTICAL METHODS

The primary objective of this Phase 2 study is to evaluate the safety and tolerability of up to 24 weeks of AG-348 in patients with PK deficiency. Therefore, analyses will be primarily descriptive in nature; no formal hypothesis testing will be conducted. All analyses will be conducted separately within each dose arm, or pooled where appropriate.

## 12.1. Sample Size Estimation

Due to the rare disease setting, the minimal sample size in each dose arm may be determined by feasibility. In order to evaluate the primary objective of safety and tolerability of AG-348 in Arm 1 and Arm 2, up to a maximum of 25 patients may be randomized onto each arm. The actual number of patients enrolled into Arms 1 and 2 will depend on the safety reviews and decisions made by the DRT. In addition, up to 25 additional patients may be enrolled to evaluate an additional dose arm (Arm 3; see Section 7.1). As for Arms 1 and 2, the actual enrollment in a potential Arm 3 will depend on the safety reviews and decisions made by the DRT. Therefore, up to approximately 75 total patients may be enrolled in this study across 2 to 3 dose arms.

Table 4 provides the probability within a dose arm of detecting 1 or more AEs with varying sample size and the true underlying AE rates.

**Table 4: Sample Size Estimation**

| Sample Size | True Underlying AE Rate | | |
|---|---|---|---|
| | **15%** | **10%** | **5%** |
| 5 | 56% | 41% | 23% |
| 10 | 80% | 65% | 40% |
| 15 | 91% | 79% | 54% |
| 25 | 98% | 93% | 72% |

## 12.2. Populations for Analysis

The following patient populations (i.e., analysis sets) will be evaluated and used for presentation of the data:

- Safety Analysis Set: All patients who are enrolled and receive any dose of study treatment. The Safety Analysis Set will be the primary set for the analysis of safety data. Patients will be classified according to treatment received, where treatment received is defined as the assigned treatment if it is received at least once, or as the first treatment received if assigned treatment is never received. Unless otherwise stated, the Safety Analysis Set will be the default analysis set for all data analyses.
- Pharmacokinetic (PK) Analysis Set: All patients in the Safety Analysis Set with sufficient plasma sample data to assess PK parameters. Results of the potential PD activity of AG-348 will also be based on the PK analysis set.

- Efficacy Analysis Set: All patients who are enrolled and achieve at least 50% compliance with their assigned dose intensity for at least 4 weeks of continuous dosing. Additional efficacy analyses may be performed on subsets of patients with other degrees of compliance with their assigned dose intensity and/or duration of dosing. The Efficacy Analysis Set will be the primary set for the analysis of preliminary clinical activity data. Patients will be classified according to assigned treatment.

[redacted] If such analyses are performed, they will be described in a separate PK Statistical Analysis Plan (SAP) and may be reported separately in a stand-alone report.

## 12.3. Procedures for Handling Missing, Unused, and Spurious Data

No imputation will be performed for missing data elements. When summarizing AE data, partial dates will be imputed as described in the SAP. Additional rules addressing the handling of missing data will be detailed in the SAP.

## 12.4. Interim Analysis

No formal statistical interim analysis will be conducted. Safety data will be reviewed on an ongoing basis by the DRT, who will meet to review safety, PK, PD, and preliminary clinical activity data at regular intervals (approximately every 6 weeks) throughout the duration of the study. The DRT's decisions to suspend, terminate, or open a potential third dosing arm, or re-assign patients' dosing in a terminated arm to match the dose and schedule of another arm of the study will be based on the totality of the data including, safety, PK, PD, and preliminary clinical activity (e.g., changes in Hb levels).

Additional interim reviews of data may be conducted to support decision making concerning the current clinical study, the sponsor's development programs in general, or in case of any safety concerns.

## 12.5. Statistical Methodology

### 12.5.1. General Methods

This study will be primarily descriptive in nature; therefore, there will be no formal hypothesis testing. Summaries will be produced for disposition, baseline disease characteristics and demographic data including genotype, safety measurements, PK, PD parameters and indicators of clinical activity. Data from each AG-348 dose group will be analyzed separately, and pooled across all dose groups where appropriate. All data will also be listed by individual patient.

Categorical variables will be summarized by frequency distributions (number and percentages) and continuous variables will be summarized by descriptive statistics (mean, standard deviation [SD], median, minimum, and maximum).

### 12.5.2. Disposition

A summary of the disposition of patients will be presented, including the number enrolled, the number treated, and the reasons for study discontinuation. Entry criteria and protocol deviations will be listed.

### 12.5.3. Exposure and Safety Analyses

Patients will receive multiple PO doses of AG-348 over a 24-week treatment period. The actual dose and duration in days of AG-348, as well as the dose intensity (computed as the ratio of actual dose received and actual duration) and the relative dose intensity (computed as the ratio of dose intensity and planned dose received/planned duration) will be listed and summarized using descriptive statistics by dose arm.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and the incidence of TEAEs (new or worsening from baseline) will be summarized by primary system organ class (SOC), preferred term (PT), CTCAE Version 4.03 severity, outcome, action taken with study drug, and relationship to the study drug by dose group. Separate summaries will be produced for all TEAEs, treatment-related AEs (those considered by the Investigator as at least possibly drug related), SAEs, discontinuations due to AEs, and AEs ≥ Grade 3 severity. Individual patient listings will be provided for deaths, SAEs, AEs leading to interruption and/or reduction of study drug dose, and AEs leading to discontinuation of treatment.

Analyses of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined as any AE with onset beginning at the day of first administration of study drug, throughout the treatment period until 30 days after the last dose of study drug, or any event that was present at baseline but worsened in intensity or was subsequently considered drug-related by the Investigator through the end of the study.

Descriptive statistics will be provided for clinical laboratory values (e.g., hematology, serum chemistry, coagulation studies, urinalysis) and VS data, presented as both actual values and changes from baseline relative to each on-study evaluation. Shift analyses will be conducted for selected laboratory parameters based on the baseline CTCAE grade to maximum CTCAE grade. Where applicable CTCAE terms do not exist, a grading system based on the upper and/or lower limits of normal will be used to classify the results.

No formal hypothesis-testing analysis of AE incidence rates will be performed.

Electrocardiogram analyses will include individual patient listings and summaries of abnormal and clinically significant ECG results. Actual values and changes from baseline in PR, QRS, and heart-rate corrected QT interval (QTc) intervals will be summarized by visit and dose arm. Full details of the QTc analysis including correction methods used will be described in the SAP.

Data collected from the menstrual diaries such as the start and stop dates of the menses and the patient reported characteristics of the menses will be presented in a by-patient listing. Additional descriptions of the data may also be performed.

Concomitant medications will be coded using the WHO Drug Dictionary. Concomitant medications prior to and after the start of the study drug will be listed by patient, and summarized by ATC term and dose arm.

### 12.5.4. Pharmacokinetic Analyses

Descriptive statistics will be used to summarize PK parameters for the parent compound AG-348 and the metabolite AGI-8702 for each dose group, and where appropriate, for the entire population. Pharmacokinetic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum and maximum, geometric mean, and geometric coefficient of variation. PK analyses will be described in a separate PK SAP.

### 12.5.5. Pharmacodynamic Analyses

Descriptive statistics will be used to summarize PD parameters for 2,3-DPG and ATP for each dose group, and where appropriate for the entire population. Pharmacodynamic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation %. PD analyses will be described in a separate PD SAP.

### 12.5.6. Aromatase Hormone Analysis

The analyses of serum sex hormones will use appropriate graphic displays and statistical analyses to evaluate patient change from baseline for each parameter. These analyses will include summaries of actual values and change from baseline using appropriate descriptive statistics (mean, SD, median, min and max) as described above.

[redacted] These analyses will present information by each dose arm, and analyses of a pooled AG-348 cohort. Additional details regarding these analyses will be provided in the SAP.

### 12.5.7. Clinical Activity

Details on analyses to evaluate indicators of potential clinical activity of AG-348 in patients with PK deficiency will be described in the SAP. These will include changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation (serum iron/iron binding capacity). Characterization of Hb response which will include, but is not limited to percent of patients with increase in Hb, time to Hb response, and duration of Hb response will be explored.

[redacted]

## 12.6. Procedures for Reporting Deviations to Original Statistical Analysis Plan

All deviations from the original SAP will be provided in the final clinical study report.

# 13. ADMINSTRATIVE REQUIREMENTS

## 13.1. Good Clinical Practice

The study will be conducted in accordance with the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s). The Investigator will be thoroughly familiar with the appropriate use of the study drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study, and retained according to the appropriate regulations.

## 13.2. Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki (Appendix 15.5).

The Investigator must obtain IRB approval for the investigation and must submit written documentation of the approval to the Sponsor before he or she can enroll any patient into the study. The IRB will review all appropriate study documentation in order to safeguard the rights, safety, and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, Investigator's Brochure, informed consent, advertisements (if applicable), written information given to the patients, signed Dose Escalation Interim Safety Reports, annual progress reports, and any revisions to these documents will be provided to the IRB. The IRB is to be notified of any amendment to the protocol in accordance with local requirements. Progress reports and notifications of serious unexpected adverse drug reactions are to be provided to the IRB according to local regulations and guidelines.

## 13.3. Patient Information and Informed Consent

The Investigator or trained designee will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk, and benefit of the study. Patients must also be notified that they are free to discontinue from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

After the study has been fully explained, written informed consent will be obtained from the patient prior to study participation.

The patient's signed and dated informed consent must be obtained before conducting any study-related procedures. The Investigator must maintain the original, signed consent form. A copy of the signed form must be given to the patient.

The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

## 13.4. Patient Confidentiality

In order to maintain patient privacy, all source documents, study drug accountability records, study reports and communications will identify the patient by the assigned patient number. The Investigator will grant monitor(s) and auditor(s) from the Sponsor or its designee and regulatory authority(ies) access to the patient's original medical records for verification of data gathered on the source documents and to audit the data collection process. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

## 13.5. Protocol Compliance

The Investigator will conduct the study in compliance with the protocol. Modifications to the protocol should not be made without agreement of both the Investigator and the Sponsor. Changes to the protocol will require written IRB approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to patients. The IRB may provide, if applicable, where regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval/favorable opinion of the IRB. The Sponsor or designee will submit all protocol modifications to the regulatory authority(ies) in accordance with the governing regulations.

When immediate deviation from the protocol is required to eliminate an immediate hazard(s) to patients, the Investigator will contact the Sponsor's Medical Monitor (or Responsible Medical Officer), if circumstances permit, to discuss the planned course of action. Any departures from the protocol must be fully documented in the source documents/database.

## 13.6. Data Management

A paper or eCRF will be completed for each patient or an electronic data capture system will be used. The electronic data capture system (EDC) (Medidata Rave®) is a software tool designed to ensure quality assurance and facilitate data capture during clinical trials. Through a system regulated workflow that includes barcode scanning and interfaces to medical equipment to avoid manual data entry, study operations performance is controlled and captured in real time. The system is fully Code of Federal Regulations (CFR) 21 Part 11 compliant. Source documentation supporting the data should indicate participation in the study and should document the dates and details of study procedures, AEs, and patient status. The Investigator, or trained designee should complete and the Investigator should verify the source documents as the information is collected. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data. The Investigator will ensure the accuracy, completeness, and timeliness of the data reported to the Sponsor. Data collection processes and procedures will be reviewed and validated to ensure completeness, accuracy, reliability, and consistency. A complete audit trail will be maintained of all data changes. The Investigator or designee will cooperate with the Sponsor's representative(s) for the periodic review of study documents to ensure the accuracy and completeness of the data capture system at each scheduled monitoring visit. Electronic consistency checks and manual review will be used to identify any errors or inconsistencies in the data. This information will be provided to the respective study sites by means of electronic or manual queries. The Investigator or designee will prepare and maintain adequate and accurate study documents (medical records, ECGs, AE, and concomitant

medication reporting, raw data collection forms, etc.) designed to record all observations and other pertinent data for each patient receiving study treatment. The Investigator will allow Sponsor representatives, contract designees, authorized regulatory authority inspectors, and the IRB to have direct access to all documents pertaining to the study.

## 13.7. Source Document/Case Report Form Completion

Source documents will be completed for each study patient. It is the Investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the patient's source document. The source document should indicate the patient's participation in the study and should document the dates and details of study procedures, AEs and patient status.

The Investigator, or designated representative, should complete the source document as soon as possible after information is collected, preferably on the same day that a study patient is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.

The Investigator will sign and date each required assessment for all study patients. The Investigator will retain all completed source documents.

## 13.8. Direct Access to Source Data

The study will be monitored by the Sponsor or its designee. Monitoring will be done by personal visits from a representative of the Sponsor (site monitor) and will include on-site review of the source documents for completeness and clarity, cross-checking with source documents, and clarification of administrative matters will be performed. The review of medical records will be performed in a manner to ensure that patient confidentiality is maintained.

The site monitor will ensure that the investigation is conducted according to protocol design and regulatory requirements by frequent communications (letter, telephone, e-mail, and fax).

All unused study drug and other study materials are to be returned to the Sponsor or designee after the study has been completed and the database has been locked.

Regulatory authorities, the IRB, and/or the Sponsor's clinical quality assurance group or designee may request access to all source documents, database, and any other applicable study documentation for an on-site audit or inspection. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities.

## 13.9. Record Retention

The Investigator will maintain all study records according to ICH-GCP and applicable regulatory requirement(s). Records will be retained for at least 2 years after the last marketing application approval or 2 years after formal discontinuation of the clinical development of the investigational product or according to applicable regulatory requirement(s). If the Investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility. The Sponsor must be notified in writing if a custodial change occurs.

## 13.10. Liability and Insurance

The Sponsor has subscribed to an insurance policy covering, in its terms and provisions, its legal liability for injuries caused to participating persons and arising out of this research performed strictly in accordance with the scientific protocol as well as with applicable law and professional standards.

## 13.11. Publication of Study Findings and Use of Information

All information regarding AG-348 supplied by the Sponsor or designee to the Investigator is privileged and confidential information. The Investigator agrees to use this information to accomplish the study and will not use it for other purposes without consent from the Sponsor. It is understood that there is an obligation to provide the Sponsor with complete data obtained during the study. The information obtained from the clinical study will be used towards the development of AG-348 and may be disclosed to regulatory authority(ies), other Investigators, corporate partners, or consultants as required.

# 14. LIST OF REFERENCES

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# 15. APPENDICES

## 15.1. Schedules of Assessments

### Table 5: Schedule of Assessments:

| **Timing** | **Pre-Treatment** | | **Month 1** | | | | **Months 2 and 3** | | | **Months 4, 5 and 6** | | | **Follow Up** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | | **Baseline / D1** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day** | **-28 to -1** | **-26 to -1**[1] | **1** | **8**[2] | **15** | **22**[2] | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window** | | | | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| Written Informed Consent | X | | | | | | | | | | | | |
| PK enzyme assay (confirmation of PK deficiency) | X | | | | | | | | | | | | |
| PKR Genotype (for randomization) | X | | | | | | | | | | | | |
| UGT1A1 Genotype | X | | | | | | | | | | | | |
| Demographics | X | | | | | | | | | | | | |
| Medical/Surgical History (General and PK deficiency-specific)[3] | X | | | | | | | | | | | | |
| Medication History | X | | | | | | | | | | | | |
| Transfusion History | X | | | | | | | | | | | | |
| Confirmation of Vaccinations (Splenectomized Patients) | X | | | | | | | | | | | | |
| Physical Examination[4]/ Height[4] and Weight | X | | X | | X | | | X | X | X | X | X | X |
| Performance Status | X | | X | | X | | | X | X | X | X | X | X |

| **Timing** | **Pre-Treatment** | | **Month 1** | | | | **Months 2 and 3** | | | **Months 4, 5 and 6** | | | **Follow Up** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | | **Baseline / D1** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day** | **-28 to -1** | **-26 to -1**[1] | **1** | **8**[2] | **15** | **22**[2] | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window** | | | | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| Vital signs (BP, HR, RR, T)[5] | X | | X | X | X | X | X | X | X | X | X | X | X |
| 12-lead ECG[6] | X | | X | X | | X | | | | | | X | X |
| DXA Scan[7] | X | | | | | | | | | | | X[8] | |
| Laboratory Evaluations[9] | | | | | | | | | | | | | |
| HBsAg, HCV Ab, HIV1 and 2 Ab | X | | | | | | | | | | | | |
| RBC antibody Screen | X | | | | | | | | | | | | |
| Hematology (CBC)[10] | X | X[11] | X | X | X | X | X | X | X | X | X | X | X |
| Haptoglobin[12] | | | X | | | X | | | X | | | X | X |
| EPO levels[13] | | | X | | | X | | | X | | | X | X |
| G6PD screen | X | | | | | | | | | | | | |
| Serum Chemistry[14] | X | | X | X | X | X | X | X | X | X | X | X | X |
| Iron Panel[15] | | | X | | | | | | X | | | X | |
| Carboxyhemoglobin (COHb) | | | X | | | X | X | X | X | X | X | X | |
| Coagulation Studies[16] | X | | X | | X | | | | X | | | X | X |
| Urinalysis[17] | X | | X | | X | | | | X | | | X | X |
| Serum or Urine Pregnancy[18] | X | | X | | | | | | | | | | |

| **Timing** | **Pre-Treatment** | | **Month 1** | | | | **Months 2 and 3** | | | **Months 4, 5 and 6** | | | **Follow Up** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | | **Baseline / D1** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day** | **-28 to -1** | **-26 to -1**[1] | **1** | **8**[2] | **15** | **22**[2] | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window** | | | | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| Lipids[19] | | | X | | | | X | | X | | | X | X |
| Hormonal Testing[20] | X | X[21] | X | | | | | | X | | | X | X |
| Serum osteocalcin-N-mid and CTX[22] | | | X | | | | | | X | | | X | |
| 25-hydroxy Vitamin D2 and D3 | | | X | | | | | | X | | | X | |
| Randomization[23] | X | | | | | | | | | | | | |
| Study Drug Administration | | | X | X | X | X | X | X | X | X | X | X | |
| Dispense Study Drug[24] | | | X | X | X | X | X | X | X | X | X | | |
| PK blood sampling[25] | | | X | | X | X | X | X | X | X | X | X | |
| PD Assessments[25] | | | | | | | | | | | | | |
| 2,3-DPG/ATP | | | X | | X | X | X | X | X | X | X | X | |
| [redacted] | | | | | | | | | | | | | |
| Dispense/Collect Menstrual Cycle Diary[27] | | | X | | | | X | | X | X | X | X | X |
| Adverse Events[28] | | | Continuous | | | | | | | | | | X |
| Transfusion Record | X | | X | X | X | X | X | X | X | X | X | X | X |

| Timing | Pre-Treatment | | Month 1 | | | | Months 2 and 3 | | | Months 4, 5 and 6 | | | Follow Up |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | | **Baseline / D1** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day** | **-28 to -1** | **-26 to -1**[1] | **1** | **8**[2] | **15** | **22**[2] | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window** | | | | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| Concomitant Medications/Procedures | X | | X | X | X | X | X | X | X | X | X | X | X |
| Rollover to safety extension | | | | | | | | | | | | X | |

Abbreviations: Ab = antibody; ATP = adenosine triphosphate; BP = blood pressure; CBC= complete blood count; COHb = carboxyhemoglobin; CTX = C-terminal telopeptide; D = day; DPG = diphosphoglycerate; DXA = Dual-energy x-ray absorptiometry; ECG = electrocardiogram; EPO = erythropoietin; FSH = follicle stimulating hormone; G6PD = glucose-6-phosphate-dehydrogenase; HBsAg = hepatitis B surface antigen; HCV Ab = hepatitis C virus antibody; HDL-C = high-density lipoprotein-C; HIV = human immunodeficiency virus; HR = heart rate; PD = pharmacodynamic; PK = pharmacokinetic; PK deficiency = pyruvate kinase deficiency; PKR = pyruvate kinase isoform R; RR = resting rate; W = week.

*Whenever more than one assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (e.g., VS, ECG, blood draw). The timing of these assessments should allow the blood draw to occur at the exact nominal time. The order of procedures may be revised with prior discussion between Sponsor and site.*

[1] To be performed at least 2 days after the first Screening Visit.

[2] In-clinic visits on Day 8 and Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis; in these instances PK/PD sampling would not be required and dispensing of study medication would not be performed. For the Day 8 visit performed by the patient's primary care physician, the primary care medical office will collect a blood sample for hematology using the blood sample collection and shipping supplies from the kit prepared by the designated central laboratory. The kit will be sent to the primary care physician's office. No other testing or procedures will be asked of the primary care physician on Day 8 [VS and serum chemistry will not be required]. The 12-lead ECG scheduled for Day 8 will instead be performed at the main study center on Day 15. For the Day 22 visit performed by the patient's primary care physician, the primary care medical office will collect a blood sample for hematology using the blood sample collection and shipping supplies from the kit prepared by the designated central laboratory. The kit will be sent to the primary care physician's office. No other testing or procedures will be asked of the primary care physician on Day 22. [VS, serum chemistry, coagulation, haptoglobin, EPO level, carboxyhemoglobin, and PK/PD samples will not be required.] The 12-lead electrocardiogram scheduled for Day 22 will instead be performed at the main study center on Day 43 (Week 6). For patients having their Day 8 and/or Day 22 visits performed by the primary care physician, the Principal Investigator will him or herself, or have a qualified research nurse or other designated site staff member, make telephone contact with the patient to inquire about any adverse events. These must be recorded as if the patient appeared in the main study center. The Principal Investigator will exercise prudent clinical judgment in determining any clinical course of action to take based on any adverse events discovered. These telephone contacts on Day 8 and Day 22 must be explained to the patient in advance and scheduled in advance to maximize the likelihood of successfully making contact.

[3] Medical history, including all relevant prior medical history, current medical conditions, and hematology profile (CBCs) over prior 6 months, will be obtained at the Screening assessment.

[4] A complete physical examination (including neurological examination; genital and rectal examinations will be performed at the discretion of the Investigator) will be obtained at Screening, Baseline, Week 12, and the Follow up Visit (Week 28), or Week 24 for patients rolling over to the safety extension. The neurological examination must include an assessment of general orientation and mental status including level of alertness (assess as normal or abnormal and specify any abnormality). Limited focused physical examinations will be performed at all other specified visits. Height to be collected at Screening only.

[5] Vital signs will include systolic and diastolic blood pressure, heart rate, respiratory rate, and temperature.

[6] 12-lead ECGs are to be conducted after 5 minutes of recumbency. Screening ECG will be performed at least 7 days prior to Day 1 dosing.

[7] If a DXA scan of the hip and spine with T and Z scores has been performed within 3 months preceding the first day of dosing, this may be used to meet this requirement.

[8] Week 24 DXA scan may be performed anytime between Weeks 24 and 28 and must be performed at the same imaging center on the same DXA machine as the original Screening DXA scan.

[9] Laboratory evaluations (hematology, serum chemistry, coagulation studies, and urinalysis) are to be collected in the morning. These should be collected following an overnight fast on Baseline Day 1 Week 6 (Day 43), Week 12 (Day 85), Week 24 (Day 169), and Follow-up Week 28 (Day 197), when the lipid samples are also included.

[10] Three Screening/Baseline samples will be collected for complete blood count (CBC). Samples will be collected in the AM on 3 different days; the samples collected on Baseline/Day 1 may comprise one of these; samples may be taken at the same time as Screening/Baseline hormone assessments (any 2 time points during Screening at least 2 days apart). CBC will include HCT, Hb, red blood cell (RBC) count, absolute reticulocyte count, percent reticulocyte count, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count with automated (or manual, as indicated) differential, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and platelet count. In the event that the designated central laboratory for the study is unable to provide a valid result for any specific component of the defined CBC for a specific patient, the site may be asked to have the test performed at their local laboratory. The result of the local CBC will be entered into the study database along with the local normal reference range.

[11] The second Screening hematology (CBC) should be drawn in the morning (does not have to be fasting), and may be drawn at the same time the patient returns for the second estradiol and free and total testosterone sample.

[12] Haptoglobin will be performed prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

[13] Erythropoietin (EPO) levels will be performed prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

[14] Alkaline phosphatase, sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide (CO2) or bicarbonate, albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and indirect bilirubin, (and estimated creatinine clearance or glomerular filtration rate for screening only, as appropriate).

[15] Iron (Fe), total iron-binding capacity (TIBC), transferrin saturation, and ferritin will be performed prior to dosing on Day 1, at the end of Week 12 and at the end of Week 24.

[16] Fibrinogen, activated partial thromboplastin time (aPTT), and international normalized ratio (INR) will be performed at Screening, prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

[17] Color, appearance, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, and occult blood. Microscopic inspection of sediment should only be performed for cause or to investigate an abnormal dipstick finding per the Investigator's discretion. Urinalysis will be performed at Screening, prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

[18] Must be repeated at any point throughout the study period if pregnancy is clinically suspected.

[19] Total cholesterol, HDL-C, and triglyceride samples will be collected in the morning following an overnight fast.

[20] Three Screening/Baseline samples will be collected for estradiol and testosterone (total and free). Samples will be collected in the AM on 3 different days; the samples collected on Baseline/Day 1 may comprise one of these; the Screening samples may be collected at any 2 time points during Screening at least 2 days apart. Serum estrone, estradiol, and free and total testosterone will then follow the schedule indicated on Day 1 and Weeks 12, 24, and 28. FSH will only be performed at Screening for female patients only for confirmation of post-menopausal status.

[21] The second Screening hormone testing will consist of estradiol and testosterone (free and total) only. Samples should be drawn in the AM (does not need to be fasting), and may be drawn at the same time the patient returns for the second CBC sample.

[22] Serum osteocalcin-N-mid and CTX will be drawn in the AM each time, approximately between 8-10 AM, and after an overnight fast of 10-12 hours.

[23] Randomization will be performed following PKR genotyping and prior to and as close as feasible to dosing on Day 1.

[24] Study drug will be dispensed on a 28-day schedule, or on an alternate schedule (< 28 days) as needed to accommodate patient visit schedule and dose modifications.

[25] For the first 10 patients treated, extensive PK/PD sampling will be conducted on Days 1 and 15 (see Appendix 15.1, Table 6 for details), followed by limited PK/PD sampling from Week 3 to Week 24 (see Appendix 15.1, Table 7 for details). Limited PK/PD sampling will be conducted on the remainder of patients treated (see Appendix 15.1 , Table 7). See Section 10.6.1, Section 10.7, and Section 10.9 for details on blood sampling for PK and PD assessments, respectively, and guidelines on sample processing and storage.

[27] Menstruating female patients will record their menstrual cycles (start, stop, characteristics) monthly. Paper-based menstrual cycle diaries will be dispensed at study visits approximately every month. The previous month's diary will be collected at these visits as well.

[28] All randomized patients will be evaluated for AEs from the time they sign informed consent until they complete all scheduled study follow-up visits or withdraw consent, whichever occurs first.

**Table 6: Schedule of Assessments: Extensive PK/PD Sampling**

| **Sample Timing/Interval** | **Month 1** | | | | | | | | **Months 2 and 3** | | | **Months 4, 5 and 6** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Baseline / D1 W2 / D15** | | | | | | | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** |
| **Study Day** | **1/15** | | | | | | | **22** | **43** | **64** | **85** | **113** | **141** | **169** |
| **Visit Window** | **± 2 D (D15)** | | | | | | | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| **Timing** | Pre-dose[1] | 30 min[2] | 1 hr[2] | 2 hr[2] | 4 hr[3] | 8 hr[3] | 12 hr[3] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] |
| PK blood sample | X | X | X | X | X | X | X[4] | X | X | X | X | X | X | X |
| 2,3 DPG/ATP | X | X | X | X | X | X | X[4] | X | X | X | X | X | X | X |
| [redacted] | | | | | | | | | | | | | | |

Abbreviations: ATP = adenosine triphosphate; D = day; DPG = diphosphoglycerate; PD = pharmacodynamic; PK = pharmacokinetic; PKR = pyruvate kinase isoform R; W = week.

[1] The acceptable time window will be within 60 minutes prior to study treatment dose administration for the pre-dose PK/PD sample.

[2] The acceptable time window will be within ± 5 minutes of the scheduled collection time for the 30 minute, 1 and 2 hour PK/PD samples.

[3] The acceptable time window will be within ± 30 minutes of the scheduled collection time for the 4, 8, and 12 hour PK/PD samples.

[4] To be collected on Day 1 only.

[redacted]

[6] If the 12 hour time point cannot be collected at site on Day 1, an 8 hour time point may be collected instead.

**Table 7: Schedule of Assessments: Limited PK/PD Sampling**

| Sample Timing/Interval | Month 1 | | | Months 2 and 3 | | | Months 4, 5 and 6 | | |
|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Baseline / D1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** |
| **Study Day** | **1** | **15** | **22** | **43** | **64** | **85** | **113** | **141** | **169** |
| **Visit Window** | **-** | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| **Timing** | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] |
| PK blood sample | X | X | X | X | X | X | X | X | X |
| 2,3 DPG/ATP | X | X | X | X | X | X | X | X | X |
| [redacted] | | | | | | | | | |

Abbreviations: ATP = adenosine triphosphate; D = day; DPG = diphosphoglycerate; PD = pharmacodynamic; PK = pharmacokinetic; PKR = pyruvate kinase isoform R; W = week.

[1] The predose blood sample for plasma PK/PD analysis should be collected within 60 minutes prior to study treatment dose administration.

## 15.2. Eastern Cooperative Oncology Group Performance Status Scoring

| Grade | Symptomatology |
|---|---|
| 0 | Fully active, able to carry on all pre-disease performance without restriction |
| 1 | Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work |
| 2 | Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours |
| 3 | Capable of only limited self-care, confined to bed or chair more than 50% of waking hours |
| 4 | Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair |
| 5 | Dead |

Source: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, et al. Toxicity and Response Criteria of The Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-655.

## 15.3. National Cancer Institute Common Terminology Criteria for Adverse Events

The NCI CTCAE, Version 4.03, can be accessed using the following link:

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

## 15.4. Potential Drug Interactions

Strong inhibitors of CYP3A4 (listed in Table 8) are not permitted for use with AG-348. Based on modeling of AG-348 metabolism and tolerability data from the Phase 1 MAD study, it is thought that moderate inhibitors of CYP3A4 do not pose a risk to patients dosed with AG-348.

In-vivo data from the Phase 1 MAD show AG-348 induces CYP3A4 and induces its own metabolism, presumably via CYP3A4. The use of strong CYP3A4 inducers in combination with AG-348 is expected to reduce AG-348's efficacy. Therefore, administration of strong CYP3A4 inducers (listed in Table 9) with AG-348 is not permitted. Strong inhibitors of drug transport (listed in Table 10) are not permitted for use with AG-348.

Digoxin is not permitted for use with AG-348.

Induction of CYP3A4 by AG-348 is expected to reduce the efficacy of certain sensitive co-medications. Co-administration of AG-348 with the drugs in Table 11 should be done with caution, as their efficacy may be reduced.

Of note, in accordance with Inclusion Criteria 14, women in the trial utilizing oral contraception must utilize barrier methods while taking AG-348.

The expected patient co-medications deferoxamine, deferasirox, deferiprone, and oral penicillin are not expected to interact with AG-348.

**Table 8: Strong and Moderate CYP3A4 Inhibitors**

| Strong CYP3A4 Inhibitors: Contraindicated | Moderate CYP3A4 Inhibitors: No Action |
|---|---|
| Indinavir | Aprepitant |
| Nelfinavir | Erythromycin |
| Ritonavir | Fluconazole |
| Clarithromycin | Verapamil[1] |
| Itraconazole | Diltiazem[1] |
| Ketoconazole | |
| Nefazodone | |
| Saquinavir | |
| Suboxone | |
| Telithromycin | |
| Grapefruit juice[2] | |

Strong Inhibitor; > 5 fold increase in AUC

Moderate Inhibitor; > 2 fold, < 5 fold increase in AUC

[1] Verapamil and diltiazem are contraindicated because they are strong P-gp inhibitors (see Table 10)

[2] Although classified as a moderate CYP3A4 inhibitor, grapefruit and grapefruit juice are prohibited

**Table 9: Strong CYP3A4 Inducers**

| **Strong CYP3A4 Inducers: Contraindicated** | |
|---|---|
| Efavirenz | Phenytoin |
| Nevirapine | Pioglitazone |
| Carbamazepine | Rifabutin |
| Glucocorticoids[1] | Rifampin |
| Modafinil | St. John's Wort |
| Oxcarbazepine | Troglitazone |
| Phenobarbital | |

[1] Short-term (≤ 14 days at a time, and ≤ 28 days total during the 24 week treatment period) use of topical, inhaled, intra-nasal, and systemic glucocorticoids is permitted for acute medical indications. Every effort should be made to minimize total duration of glucocorticoid therapy and utilize alternative treatments. Patients must be off glucocorticoids for at least 28 days prior to Day 1 of AG-348 dosing as per exclusion criterion #11 (Section 8.3). For patients who require chronic inhaled glucocorticoid therapy, Investigators should confer with the Medical Monitor for additional guidance.

**Table 10: Strong P-glycoprotein Inhibitors**

| **Strong P-gp Inhibitors: Contraindicated** | |
|---|---|
| Amiodarone | Felodipine |
| Azithromycin | Itraconazole |
| Captopril | Ketoconazole |
| Carvedilol | Lopinavir |
| Clarithromycin | Ritonavir |
| Conivaptan | Quercetin |
| Cyclosporine | Quinidine |
| Diltiazem | Ranolazine |
| Dronedarone | Ticagrelor |
| Erythromycin | Verapamil |

**Table 11: Sensitive CYP3A4 Substrates**

| **Sensitive CYP3A4 Substrates: Substitute or Use with Caution** | | | | |
|---|---|---|---|---|
| **Macrolide antibiotics:** | **Antihistamines:** | **Miscellaneous:** | | |
| Erythromycin | Chlorpheniramine | Alfentanil | Finasteride | Salmeterol |
| | | Aprepitant | Gleevec | Sildenafil |
| **Benzodiazepines:** | **Calcium Channel Blockers:** | Aripiprazole | Haloperidol | Sirolimus |
| Alprazolam | Amlodipine | Boceprevir | Irinotecan | Sorafenib |
| Diazepam→3OH | Lercanidipine | Buspirone | LAAM | Sunitinib |
| Midazolam | Nifedipine | Cafergot | Lidocaine | Tamoxifen |
| Triazolam | Nisoldipine | Caffeine→TMU | Methadone | Taxol |
| | Nitrendipine | Cilostazol | Nateglinide | Telaprevir |
| **Immune Modulators:** | | Cocaine | Nevirapine | Terfenadine |
| Tacrolimus (FK506) | **HMG CoA Reductase Inhibitors**: | Codeine-N-demethylation | Ondansetron | Torisel |
| | Atorvastatin | Dapsone | Pimozide | Trazodone |
| **Steroid 6beta-OH:** | Cerivastatin | Dextromethorphan | Propranolol | Vemurafenib |
| Estradiol | Lovastatin | Docetaxel | Quetiapine | Vincristine |
| Hydrocortisone (and other glucocorticoids) | Simvastatin | Domperidone | Quinine | Zaleplon |
| Progesterone | | Eplerenone | Risperidone | Ziprasidone |
| Testosterone | | Fentanyl | Romidepsin | Zolpidem |

## 15.5. Declaration of Helsinki

**WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI:**

Recommendations Guiding Medical Doctors in Biomedical Research Involving Human Subjects

Adopted by the 18th World Medical Association (WMA) General Assembly, Helsinki, Finland, June 1964 and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975, 35th WMA General Assembly, Venice, Italy, October 1983, and the 41st WMA General Assembly, Hong Kong, September 1989, the 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996; 52nd WMA General Assembly, Edinburgh, Scotland, October 2000; 53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added); 55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added); and 59th WMA General Assembly, Seoul, October 2008.

**A. INTRODUCTION**

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.
   The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of subjects, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my subject will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the subject's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
8. In medical practice and in medical research, most interventions involve risks and burdens.

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.
10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

**B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH**

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.
12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, Sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.
15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the Sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any SAEs. No change to the protocol may be made without consideration and approval by the committee.
16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on subjects or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects

must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.
25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.
27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.
28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious subjects, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.
30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

## C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the subjects who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:
    - The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
    - Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the subjects who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.
33. At the conclusion of the study, subjects entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.
34. The physician must fully inform the subject which aspects of the care are related to the research. The refusal of a subject to participate in a study or the subject's decision to withdraw from the study must never interfere with the subject-physician relationship.
35. In the treatment of a subject, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the subject or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

agios

# Clinical Study Protocol AG348-C-003
# EudraCT No. 2015-000484-13
# A Phase 2, Open Label, Randomized, Dose Ranging, Safety, Efficacy, Pharmacokinetic and Pharmacodynamic Study of AG-348 in Adult Patients with Pyruvate Kinase Deficiency

| | |
|---|---|
| Study Sponsor: | Agios Pharmaceuticals, Inc.<br>88 Sidney Street<br>Cambridge, MA 02139-4169<br>Phone: 617-649-8600<br>Fax: 617-649-8618 |
| Responsible Medical Officer: | [redacted], MD<br>[redacted]<br>Agios Pharmaceuticals, Inc.<br>Mobile Phone: [redacted]<br>Office Phone: [redacted]<br>Email: [redacted] |
| Study Medical Monitor | [redacted], MD<br>[redacted]<br>On behalf of Agios Pharmaceuticals, Inc.<br>Mobile Phone: [redacted]<br>Office Phone: [redacted]<br>Email: [redacted] |
| Document Version (Date):<br>Revised | Version 1.0 (05 January 2015)<br>Amendment 1, Protocol Version 2.0 (02 February 2015) Final |

This study will be conducted according to the protocol and in compliance with Good Clinical Practice, the ethical principles stated in the Declaration of Helsinki, and other applicable regulatory requirements.

**CONFIDENTIALITY NOTE:**

The information contained in this document is confidential and proprietary to Agios Pharmaceuticals, Inc. Any distribution, copying, or disclosure is strictly prohibited unless such disclosure is required by federal regulations or state law. Persons to whom the information is disclosed must know that it is confidential and that it may not be further disclosed by them.

# INVESTIGATOR'S AGREEMENT

I understand that all documentation provided to me by Agios or its designated representative(s) concerning this study that has not been published previously will be kept in strict confidence. This documentation includes the study protocol, Investigator's Brochure, case report forms, and other scientific data.

This study will not commence without the prior written approval of a properly constituted Institutional Review Board (IRB). No changes will be made to the study protocol without the prior written approval of Agios and the IRB, except where necessary to eliminate an immediate hazard to the patient.

I have read, understood, and agree to conduct this study as outlined in the protocol and in accordance with the guidelines and all applicable government regulations.

| Investigator Name (printed) | Investigator Signature | Date |
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Investigational site or name of institution and location (printed)

# 2. SYNOPSIS

**Name of Sponsor/Company:**
Agios Pharmaceuticals, Inc.

**Name of Investigational Product:**
AG-348

**Name of Active Ingredient:**
AG-348 sulfate hydrate

**Title of Study:**
A Phase 2, Open Label, Randomized, Dose Ranging, Safety, Efficacy, Pharmacokinetic and Pharmacodynamic Study of AG-348 in Adult Patients with Pyruvate Kinase Deficiency

**Study Center(s):**
This study will be conducted at multiple study centers.

**Phase of Development:** 2

***Objectives:***

**Primary:**

- Evaluate the safety and tolerability of up to 24 weeks of AG-348 administration in patients with pyruvate kinase deficiency (PK deficiency).

**Secondary:**

- Evaluate the pharmacokinetics (PK) of AG-348 and the metabolite AGI-8702.
- Evaluate the pharmacodynamic (PD) response of adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG) after administration of AG-348.
- Evaluate indicators of clinical activity of AG-348 in patients with PK deficiency, including changes in hemoglobin (Hb), hematocrit (HCT), reticulocyte count, haptoglobin (Hp), carboxyhemoglobin (COHb), lactate dehydrogenase (LDH), total and indirect bilirubin, erythropoietin (EPO), ferritin, and transferrin saturation (serum iron/iron binding capacity).

[redacted]

**Methodology:**
Study AG348-C-003 is a Phase 2, open label, two arm, multicenter, randomized, dose-ranging study during which adult patients with PK deficiency will receive multiple doses of AG-348 for up to 24 weeks. Patients with PK deficiency confirmed by red blood cell PK enzymatic assay performed at Screening will be eligible to participate in this study. At Week 25, patients who safely tolerate AG-348 and demonstrate clinical activity of AG-348 may be eligible to immediately roll over to a separate safety extension study for continued treatment. Patients who finish treatment at the end of 24 weeks or sooner will undergo follow-up assessment 4 weeks after the last dose of study drug. Patients with toxicity suspected to be related to study drug will continue follow-up until the adverse event (AE) resolves, is declared chronic by the Investigator, or the patient is lost to follow-up.

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Initially, up to 25 patients will be randomized on an open-label 1:1 basis to each of two twice-daily (BID) doses of AG-348 (up to 50 patients total; see Figure 1, Study Schema). The dose of Arm 1 is 300 mg of AG-348 administered orally every 12 hours (q12h, BID). The dose of Arm 2 is 50 mg of AG-348 administered orally q12h (BID). Randomization will be stratified by *PKR* mutation in order to maintain balance as much as possible across the dose arms for the specific mutations expected to be most frequently enrolled. The *PKR* mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H) and all other mutations ("other"). Mutation status is defined by the presence of at least one of the indicated mutations; patients with more than one stratified mutation will be assigned based on Sponsor's discretion.

The doses for each arm have been selected from the forerunner AG348-C-001 single ascending dose (SAD) and AG348-C-002 multiple ascending dose (MAD) studies in healthy adult volunteers to represent the range of doses/exposures that were safely tolerated and resulted in maximal or near-maximal PD effects on 2,3-DPG and ATP.

Because PK deficiency is a rare disease with a limited eligible patient population and because the underlying pathophysiology and clinical phenotype of affected patients is heterogenous due to the wide variety of mutations in PKR that cause the disease, it is important to focus closely on dose findings in this first-in-patient study. Therefore, in addition to initiating this study with 2 different doses of AG-348 administered q12h, a Data Review Team (DRT) will be established to review study data on a regular basis and adapt the study design, dose and schedule of AG-348.

The DRT will monitor safety on an on-going basis and meet at regular intervals (approximately every 6 weeks), or *ad* hoc, as necessary, to review AEs, vital signs (VS), clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and electrocardiograms (ECGs) on enrolled patients. The DRT will also review available PK/PD data and indicators of clinical activity (e.g., changes from baseline in Hb). The DRT will be comprised of the study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer.

Approximately every 6 weeks beginning six weeks after the first patient is dosed or *ad hoc* as necessary, the DRT will review cumulative safety data, available PK/PD data, and clinical activity data. Based on the DRT's recurring 6 week reviews, the DRT may exercise one or more of the following options:

- Continue treatment and enrollment in existing arms without change.
- Add 1 new dose arm (Arm 3) to enroll up to 25 patients at a dose to be determined; the dose for Arm 3 may be lower or higher than Arm 1 and Arm 2 doses, but will not exceed 360 mg q12h; and the dose regimen may be less frequent than q12h.
- Terminate or suspend enrollment to allow further review of clinical data in Arm 1 and/or Arm 2 (and/or potential Arm 3). Enrollment in an arm could be terminated or suspended to allow further review, for example, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity.
- Re-assign patients' doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the patients in the terminated arm will remain in their original arm, i.e., they will not count towards the enrollment quota of the arm whose dose and

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schedule is being adopted.

- Implement specific genotype restrictions to enrollment in one or more arms to ensure representation of patients with genotypes of greatest clinical relevance.

The DRT will perform these evaluations on a recurring 6-week basis. The data that the DRT will review to make these decisions is expected to include, but are not necessarily limited to, the following:

- *Safety Observations:* all AEs; VS, clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and ECG;
- *PK and PD Observations:* including changes in 2,3-DPG and ATP;
- *Indicators of Clinical Activity:* including changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, EPO, total and indirect bilirubin, ferritin, and transferrin saturation.

If a third dose arm is implemented, the dose of AG-348 selected will not exceed 360 mg BID, as this was the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy volunteers. The PK/PD sampling schedule in a potential third dosing arm will be determined by Sponsor's discretion and may follow either the extensive or limited PK/PD sampling schedules as specified.

The DRT will monitor the data in an ongoing manner as described and may make a decision to terminate enrollment in an arm if unacceptable AEs are observed, PD activity is limited, and/or markers of clinical activity indicate no effect. Unacceptable AEs are defined as ≥ Grade 3 AEs (using National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAEv4.03]) that are assessed as at least possibly related to AG-348, with the exception of Grade 3 headache, hot flash/flushing, nausea, vomiting, and/or diarrhea that is transient in duration (< 24 hours) or able to be medically managed to ≤ Grade 2 within 24 hours. The DRT may also exercise discretion to terminate enrollment in an arm if it observes a frequency of Grade 2 AEs that would make it difficult to support long-term dosing.

Due to the potential for AG-348-mediated aromatase inhibition, dual-energy x-ray absorptiometry (DXA) scan (hip and spine) will be performed at Screening (if patient has not had prior DXA scan within 3 months of Day 1) to obtain T and Z scores. These data are intended to serve as a baseline measure of bone mineral density for all enrolling patients, and are deemed of particular importance for those who may enter the longer term safety extension study after completing the current study. All patients will have a second DXA scan in the interval between Weeks 24 and 28. The safety extension study will separately address additional follow-up DXA scanning.

As the number of enrolling arms changes in the study (for example, from 2 to 3), the randomization scheme will adjust to enable balanced randomization into each actively accruing arm. Randomization and stratification will cease in the event that only a single arm is left enrolling.

Depending on possible early termination of 1 or both of the initial 2 arms, or the addition of a third arm, the study could enroll up to a maximum of 75 patients.

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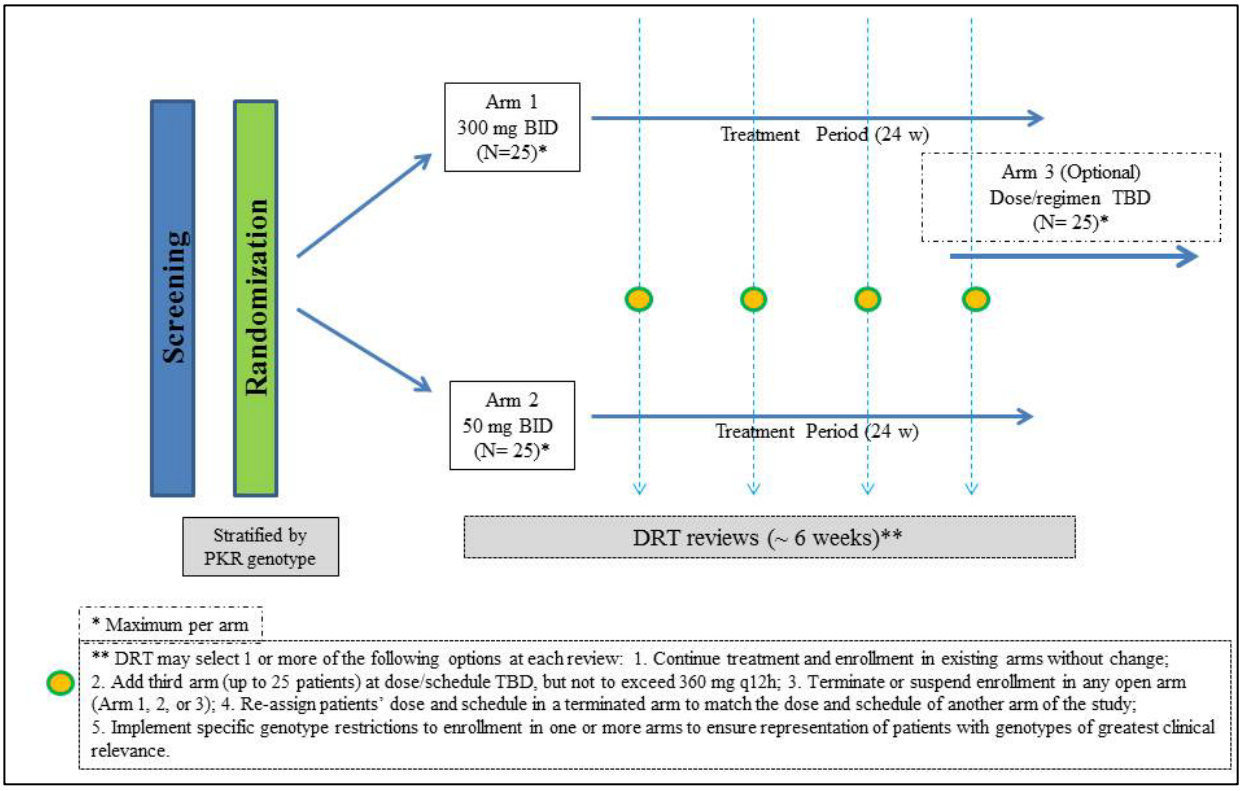
**Figure 1: Study Schema**




Abbreviations: BID (q12h) = twice-daily (every 12 hours); DRT = data review team; PKR = pyruvate kinase red blood cell isoform; TBD= to be determined; w = week(s).

***Visit Schedule***

Screening assessments will occur within 28 days prior to the first dose of study treatment. During the Treatment period, patients will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12), and monthly through Week 24 (Weeks 16, 20, and 24). Patients who safely tolerate AG-348 and demonstrate evidence of clinical activity of AG-348 through Week 24 may be eligible to immediately enter a separate extension study for continued treatment. For patients who finish treatment at the end of 24 weeks or sooner, or who elect not to enter the extension trial, study discharge will occur 4 weeks (Week 28 or earlier) following the last dose of study treatment at the final follow-up assessment.

**Dose Modifications for Safety and/or Increase in Hb Level**

The Investigator will monitor all patients for safety and tolerability. Modification of an individual patient's dose of AG-348 will be based on AEs and/or observed changes in Hb level as described in Section 9.7.1 and Section 9.7.2.

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**Number of patients (planned):** Up to approximately 75 patients.

**Diagnosis and main criteria for inclusion:**

**Inclusion criteria:**

For entry into the study, patients must meet all of the following criteria during the Screening or other specified period:

1. Signed written informed consent obtained prior to performing any study procedure, including screening procedures.
2. Male or female, aged 18 years and older.
3. Known medical history of PK deficiency.
4. All patients must have documented clinical laboratory confirmation of PK deficiency by red blood cell pyruvate kinase enzymatic assay performed at Screening by a designated central laboratory. Patients with prior documentation of PK deficiency by red blood cell (RBC) enzymatic assay will have a reconfirmation of this result during Screening as a condition of enrollment.
    a. In the event that a patient's screening pyruvate kinase enzymatic assay is negative (i.e., shows normal pyruvate kinase activity), the patient will be eligible for enrollment if the genotyping shows a mutant genotype that has been previously documented in the literature to be associated with pyruvate kinase deficiency. If the genotyping shows a previously undescribed mutation in the PKR gene, then the eligibility for enrollment will be determined on a case-by-case basis by the Coordinating Investigator and Medical Monitor in discussion with the Investigator. If no mutation is defined, then the patient will not be eligible.
5. All patients must have genotypic characterization of the mutant PKR gene performed by a designated central laboratory at Screening, unless genotype is available from the patient's participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480).
6. All patients must have genotypic characterization of the UGT1A1 gene performed by a designated central laboratory to document whether they may have underlying Gilbert's Disease. Patients with Gilbert's Disease are eligible to enroll.
7. Males must have Hb $\leq$ 12.0 g/dL; females must have Hb $\leq$ 11.0 g/dL.
8. All patients must be considered transfusion independent as defined by: no greater than 3 units of RBCs transfused in the 12-month period up to the first day of study dosing and no transfusions within 4 months of first day of study dosing. Patients who have received more transfusion support than described above will evaluated for eligibility on a case-by-case basis by the

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Medical Monitor.

9. Splenectomized patients:
    a. Must have undergone their procedure at least 6 months prior to Screening.
    b. Must be current in their vaccinations for Pneumococcal Conjugate (PCV13), Pneumococcal Polysaccharide (PPSV23), Quadrivalent Meningococcal vaccine, and *Haemophilus influenzae* type b (Hib) as recommended by Centers for Disease Control and Prevention's (CDC) Advisory Committee on Immunization Practices (ACIP) or immunization advisory groups in Canada and the European Union (for patients enrolled in Canada and the EU). [http://www.cdc.gov/vaccines/schedules/downloads/adult/adult-schedule.pdf] [Any missing vaccinations may be administered during the screening period.]
10. Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 2.
11. Patients must be taking at least 1 mg of folic acid daily for at least 21 days prior to first dose and continue daily during study participation.
12. Adequate organ function, defined as:
    a. Serum aspartate transaminase (AST) and alanine aminotransferase (ALT) ≤ 1.5 × upper limit of normal (ULN) (unless the increased AST is assessed by the Investigator as due to hemolysis).
    b. Normal or elevated levels of serum bilirubin. In patients with serum bilirubin > ULN, the elevation must be attributed to hemolysis with or without Gilbert's syndrome and must not be choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease.
    c. Serum creatinine ≤ 1.25 × ULN. If serum creatinine > 1.25 × ULN, then 24-hour measured or calculated (Cockcroft-Gault) glomerular filtration rate (GFR) ≥ 60 mL/min.
    d. Absolute neutrophil count (ANC) > $1.0 \times 10^9$/L.
    e. Platelet count ≥ $100 \times 10^9$/L.
    f. Activated partial thromboplastin time (aPTT) and international normalized ratio (INR) ≤ 1.25 × ULN, unless the patient is receiving therapeutic anticoagulants.
13. Women of childbearing potential (WOCBP) must agree to abstain from sexual intercourse or to use an acceptable/effective method of contraception (i.e., condom plus spermicide, condom plus oral contraceptive, condom plus intrauterine device [IUD], condom plus diaphragm with spermicide) from as soon as feasible during the Screening period until 30 days following the last dose of AG-348.
    a. WOCBP include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, and/or bilateral oophorectomy) or is not post-menopausal. Post-menopausal is defined as:
        i. Amenorrhea ≥ 12 consecutive months without another cause, and a documented

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| serum follicle stimulating hormone (FSH) level > 35 mIU/mL;<br>ii. Amenorrhea ≥ 12 consecutive months in women ≥ 62 years old (FSH testing is not required). |
| 14. WOCBP must have a negative serum or urine pregnancy test within 72 hours before start of AG-348 dosing.<br>15. Women must not be breastfeeding.<br>16. Male patients, with the exception of those who have undergone vasectomy at least 6 months prior to Screening, must agree to abstain from sexual intercourse or, if sexually active, to use a condom with spermicide as contraception (regardless of their female partner's childbearing potential or their partner's use of their own contraception) from Day 1 of dosing until 30 days following the last dose of AG-348. |
| **Exclusion criteria:**<br>Patients who meet any of the following criteria at Screening or prior to dosing on Day 1 will not be enrolled in the study:<br>1. Hemoglobin level > 12.0 g/dL if male; Hb > 11.0 g/dL if female.<br>2. Additional diagnosis of any other congenital or acquired blood disorder, including glucose-6-phosphate-dehydrogenase (G6PD) deficiency, or any other hemolytic anemia process except for mild allo-immunization as a consequence of transfusion therapy.<br>3. Iron overload (hemosiderosis or concurrent hemochromatosis) sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac, hepatic, or pancreatic insufficiency.<br>4. Prior bone marrow or stem cell transplant.<br>5. Clinically symptomatic cholelithiasis or cholecystitis. (Prior cholecystectomy is not exclusionary. Patients with symptomatic cholelithiasis or cholecystitis may be rescreened once the disorder has been treated and clinical symptoms have resolved.)<br>6. Currently enrolled in another therapeutic clinical trial involving on-going therapy with any investigational or marketed product or placebo. Concurrent participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480) is permitted.<br>7. Exposure to any investigational drug, device, or procedure within 28 days prior to Screening.<br>8. Concurrent medical condition that could compromise participation in the study such as:<br>a. Poorly controlled hypertension (defined as systolic blood pressure (BP) > 150 mm Hg or diastolic BP > 90 mm Hg) refractory to medical management.<br>b. History of recent (within < 6 months from Screening date) congestive heart failure; |

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myocardial infarction or unstable angina pectoris; or hemorrhagic, embolic, or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.

c. Currently active infection requiring the use of parenteral anti-microbial agents or that is greater than Grade 3 (CTCAEv4.03) within 6 months of first dose.

d. A pattern or frequency of post-splenectomy sepsis that in the assessment of the Investigator could reasonably be expected to interfere with the ability of the patient to complete the 24 week study participation.

e. Positive test for hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV) antibody with signs of active Hepatitis B or C virus infection.

f. Positive test for human immunodeficiency virus (HIV) 1 or 2 antibody.

g. Diabetes mellitus judged to be in poor control by the Investigator or requiring > 3 anti-diabetic agents counting insulin (all insulins are considered one agent); use of insulin per se is not exclusionary.

h. History of any primary malignancy with the exception of: curatively treated non-melanomatous skin cancer; curatively treated cervical or breast carcinoma in situ; or other primary tumor treated with curative intent and no known active disease present and no treatment administered during the last 3 years.

9. Undergone major surgery within 6 months of first dose.
10. Current or recent history of psychiatric disorder that in the opinion of the Investigator or Medical Monitor could compromise the ability of the patient to cooperate with study visits and procedures.
11. Use of any of the restricted list of products known to strongly inhibit CYP3A4 drug metabolism (Appendix 15.3, Table 7) within 5 days prior to Day 1 dosing; or to strongly induce CYP3A4 metabolism (Appendix 15.3, Table 8) within 28 days prior to Day 1 dosing; or to strongly inhibit P-glycoprotein (P-gp) transporter (Appendix 15.3, Table 9) within 5 days prior to Day 1 dosing; or digoxin within 5 days prior to Day 1 dosing.
12. Serum bilirubin > ULN attributable to factors other than hemolysis and/or Gilbert's syndrome.
13. Male patients with heart-rate corrected QT (Fridericia's correction factor) QTcF interval > 450 msec, or female patients with QTcF interval > 470 msec with the exception of patients with a left bundle branch block (LBBB). Medical Monitor approval needed in patients with a LBBB.
14. Cardiac dysrhythmias judged as clinically significant by the Investigator or requiring therapy with drugs that are primarily substrates of CYP3A4.

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15. History of allergy to sulfonamides if characterized by acute hemolytic anemia, anaphylaxis, or rash of erythema multiforme type or Stevens-Johnson syndrome.
16. Any other medical or psychological condition deemed by the Investigator to be likely to interfere with a patient's ability to understand and sign informed consent; cooperate with study visits, tests, and procedures; or otherwise safely and reliably participate in the study.

**Investigational product, dosage, and mode of administration:**
AG-348 sulfate hydrate capsules will be provided as 25 mg or 100 mg (free-base equivalent) of AG-348 sulfate hydrate without excipients in Swedish orange (25 mg) or white opaque (100 mg) gelatin capsules (size 2 capsules for all dose strengths). AG-348 will be administered orally BID. The number of capsules per dose will vary by assigned dose group. Patients will receive multiple oral (PO) doses of AG-348 over a 24-week treatment period. AG-348 will be administered with water and may be administered with or without food.

**Reference therapy, dosage and mode of administration:**
Not applicable.

**Duration of treatment:**
The duration of treatment for all patients on this study will be up to 24 weeks. Patients who safely tolerate and demonstrate one or more indicators of clinical activity of AG-348 may be eligible to immediately roll over to a separate safety extension study for continued treatment.

**Criteria for evaluation:**

**Safety:**
Monitoring of AEs, including determination of serious adverse events (SAEs) and AEs leading to discontinuation; safety laboratory parameters (hematology, chemistry, urinalysis, coagulation); physical examination findings; VS; 12-lead ECGs, and DXA scans. Adverse events will be graded using CTCAE, Version 4.03. Serum sex hormone levels (testosterone, estrone, and estradiol), bone turnover markers (serum N-terminal telopeptide [NTX] and serum C-terminal telopeptide [CTX]), total cholesterol, high-density lipoprotein-C (HDL-C), and triglycerides will be monitored for evidence of potential inhibition of aromatase by AG-348

**Indicators of Clinical Activity:**
Monitoring of potential indicators of clinical activity will include evaluating changes in Hb, HCT, reticulocyte count, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation.

**Pharmacokinetics:**
Approximately the first 10 patients treated, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 15.1, Table 5. The remainder of treated patients will undergo limited PK sampling as detailed in Appendix 15.1, Table 6. Serial blood sampling for determination of concentration-time profiles of AG-348 and its metabolite AGI-8702 will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of AG-348 and AGI-8702 will be obtained. AG-348 and AGI-8702 will be analyzed using qualified assays to determine concentrations in

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plasma. Pharmacokinetic parameters on Day 1 and Day 15 will be computed using standard non-compartmental methods based on observed plasma AG-348 and AGI-8702 concentrations.

**Pharmacodynamics:**

Pharmacodynamic assessments will include 2,3-DPG, ATP (secondary objectives), [redacted]. Approximately the first 10 patients treated will undergo extensive PD sampling as detailed in Appendix 15.1, Table 5. The remainder of treated patients will undergo limited PD sampling as detailed in Appendix 15.1, Table 6. Serial blood sampling for determination of levels of ATP and, 2,3-DPG will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of ATP and 2,3-DPG will be obtained. Adenosine triphosphate and 2,3 DPG will be analyzed using qualified assays to determine concentrations in whole blood. Pharmacodynamic parameters on Day 1 and Day 15 will be computed based on observed whole blood ATP and 2,3-DPG concentrations.

[redacted]

**Statistical methods:**

The primary objective of this Phase 2 study is to evaluate the safety and tolerability of up to 24 weeks of AG-348 in patients with PK deficiency. Therefore, analyses will be primarily descriptive in nature; no formal hypothesis testing will be conducted. All analyses will be conducted separately within each dose arm, or pooled when appropriate.

Summaries will be produced for disposition, baseline disease characteristics and demographic data including genotype, safety measurements, PK, PD parameters and indicators of clinical activity. Data from each AG-348 dose group will be analyzed separately, and pooled across all dose groups where appropriate. All data will also be listed by individual patient. Categorical variables will be summarized by frequency distributions (number and percentages) and continuous variables will be summarized by descriptive statistics (mean, standard deviation [SD], median, minimum, and maximum).

Populations for analysis will include a Safety Analysis Set, a PK Analysis Set, and an Efficacy Analysis Set. The Safety Analysis set will include all patients who are enrolled and receive any dose of study treatment; this population will be the primary set for the analysis of safety data and the default analysis set for all data analyses unless otherwise stated. Patients will be classified according to treatment received, where treatment received is defined as the assigned treatment if it is received at least once, or as the first treatment received if assigned treatment is never received. The PK Analysis Set will include all patients in the Safety Analysis Set with sufficient plasma sample data to assess PK parameters. Results of the potential PD activity of AG-348 will also be based on the PK analysis set. The Efficacy

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Analysis Set will include all patients who are enrolled and achieve at least 50% compliance with their assigned dose intensity for at least 4 weeks of continued dosing. Additional efficacy analyses may be performed on subsets of patients with other degrees of compliance with their assigned dose intensity and/or duration of dosing. The Efficacy Analysis Set will be the primary set for the analysis of preliminary clinical activity data. Patients will be classified according to assigned treatment. [redacted]

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and the incidence of treatment-emergent AEs (TEAEs) (new or worsening from baseline) will be summarized by primary system organ class (SOC), preferred term (PT), CTCAE Version 4.03 severity, outcome, action taken with study drug, and relationship to study drug by dose group. Separate summaries will be produced for all TEAEs, treatment-related AEs (those considered by the Investigator as at least possibly drug related), SAEs, discontinuations due to AEs, and AEs ≥ Grade 3 severity. Individual patient listings will be provided for any deaths, SAEs, AEs leading to interruption and/or reduction of study drug dose, and AEs leading to discontinuation of treatment. Analyses of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined as any AE with onset beginning at the day of first administration of study drug, throughout the treatment period until 30 days after the last dose of study drug, or any event that was present at baseline but worsened in intensity or was subsequently considered drug-related by the Investigator through the end of the study.

Descriptive statistics will be provided for clinical laboratory values (e.g., hematology, serum chemistry, coagulation studies, urinalysis) and VS data, presented as both actual values and changes from baseline relative to each on-study evaluation. Shift analyses will be conducted for selected laboratory parameters based on the baseline CTCAE grade to maximum CTCAE grade. Where applicable CTCAE terms do not exist, a grading system based on the upper and/or lower limits of normal will be used to classify the results.

Electrocardiogram analyses will include individual patient listings and summaries of abnormal and clinically significant ECG results. Actual values and changes from baseline in PR, QRS, QTc intervals will be summarized by visit and dose arm.

Concomitant medications will be coded using the WHO Drug Dictionary. Concomitant medications prior to and after the start of the study drug will be listed by patient, and summarized by ATC term and dose arm.

Descriptive statistics will be used to summarize PK parameters for the parent compound AG-348 and the metabolite AGI-8702 for each dose group, and where appropriate, for the entire population. Pharmacokinetic parameters will be summarized using the following descriptive statistics: n, mean, standard deviation (SD), coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation.

Descriptive statistics will be used to summarize Pharmacodynamic parameters for 2,3-DPG and ATP for each dose group, and where appropriate for the entire population. Pharmacodynamic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation %.

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Analyses of serum sex hormones will use appropriate graphic displays and statistical analyses to evaluate patient change from baseline for each parameter. These analyses will include summaries of actual values and change from baseline using appropriate descriptive statistics (mean, SD, median, min and max) as described above.

Analyses evaluating indicators of potential clinical activity of AG-348 in patients with PK deficiency will include changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation (serum iron/iron binding capacity). Characterization of Hb response (e.g., % of patients whose Hb increases by a certain amount), as well as time to Hb response, and duration of Hb response will be explored, among others.

**Interim Review**

No formal statistical analysis will be conducted. Safety data will be reviewed on an ongoing basis by the DRT, who will meet to review safety, PK, PD, and preliminary clinical activity data at regular intervals (approximately every 6 weeks) throughout the duration of the study. The composition of the DRT, its meeting schedule, the data to be reviewed, and the decisions it is empowered to make have been described previously.

Additional interim reviews of data may be conducted to support decision making concerning the current clinical study, the sponsor's development programs in general, or in case of any safety concerns.

**Sample Size**

Due to the rare disease setting, the minimal sample size may be determined by feasibility. Depending on possible early termination of 1 or both of the initial 2 arms or the addition of a 3$^{rd}$ dose arm, the study could enroll up to a maximum of 75 patients.

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# 4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

| Abbreviation or Specialist Term | Explanation |
|---|---|
| 2,3-DPG | 2,3-diphosphoglycerate |
| ACIP | Advisory Committee on Immunization Practices |
| ADME | Absorption, distribution, metabolism, excretion |
| ADP | Adenosine diphosphate |
| AE | Adverse event |
| ALC | Absolute lymphocyte count |
| ALP | Alkaline phosphatase |
| ALT | Alanine aminotransferase |
| ANC | Absolute neutrophil count |
| aPTT | Activated partial thromboplastin time |
| AST | Aspartate aminotransferase |
| ATP | Adenosine triphosphate |
| AUC | Area under the curve |
| $AUC_{0\text{-}12hr}$ | Area under the plasma concentration versus time curve from 0 to 12 hours |
| $AUC_{0\ 24hr}$ | Area under the plasma concentration versus time curve from 0 to 24 hours |
| $AUC_{0\text{-}\infty}$ | Area under the plasma concentration versus time curve from 0 to infinity |
| BCRP | Breast cancer resistance protein |
| BID | Twice-daily |
| BMI | Body mass index |
| BP | Blood pressure |
| BUN | Blood urea nitrogen |
| CDC | Centers for Disease Control and Prevention |
| CFR | Code of Federal Regulations |
| $CL_P$ | Total body plasma clearance |
| $C_{max}$ | Maximum plasma concentration |
| $CO_2$ | Carbon dioxide |
| COHb | Carboxyhemoglobin |
| CTCAE | Common Terminology Criteria for Adverse Events |

| Abbreviation or Specialist Term | Explanation |
|---|---|
| CTX | Serum C-terminal telopeptide |
| CV | Cardiovascular |
| DDI | Drug-drug interaction |
| CYP | Cytochrome P450 |
| DILI | Drug-induced liver injury |
| DLT | Dose-limiting toxicity |
| DRT | Data review team |
| DXA | Dual-energy x-ray absorptiometry |
| ECG | Electrocardiogram |
| ECOG | Eastern Cooperative Oncology Group |
| eCRF | Electronic case report form |
| EDC | Electronic Data Capture |
| EPO | Erythropoietin |
| F | Oral bioavailability |
| FDA | Food and Drug Administration |
| FSH | Follicle stimulating hormone |
| G6PD | Glucose-6-phosphate-dehydrogenase |
| GCP | Good Clinical Practice |
| GFR | Glomerular filtration rate |
| GLP | Good Laboratory Practice |
| Hb | Hemoglobin |
| HBsAg | Hepatitis B surface antigen |
| HCT | Hematocrit |
| HCV | Hepatitis C virus |
| HDL-C | High-density lipoprotein-C |
| HDPE | High density polyethylene |
| hERG | Human ether à-go-go related gene |
| Hib | *Haemophilus influenzae* type b |
| HIV | Human immunodeficiency virus |
| Hp | Haptoglobin |
| $IC_{50}$ | Concentration of drug that achieved half-maximal inhibition |
| ICH | International Conference on Harmonization |

| Abbreviation or Specialist Term | Explanation |
|---|---|
| INR | International normalized ratio |
| IRB | Institutional Review Board |
| IUD | Intrauterine device |
| IV | Intravenous |
| LDH | Lactate dehydrogenase |
| MAD | Multiple ascending dose |
| MCH | Mean corpuscular hemoglobin |
| MCHC | Mean corpuscular hemoglobin concentration |
| MCV | Mean corpuscular volume |
| MedDRA | Medical Dictionary for Regulatory Activities |
| mPKR | PKR mutants |
| MTD | Maximum tolerated dose |
| NCI | National Cancer Institute |
| NOEL | No-observed-effect-level |
| NOAEL | No-observed-adverse-effect-level |
| NTX | Serum N-terminal telopeptide |
| P-gp | P-glycoprotein |
| PCV13 | Pneumococcal Conjugate |
| PD | Pharmacodynamic |
| PEP | Phosphoenolpyruvate |
| PK | Pharmacokinetic |
| PK deficiency | Pyruvate kinase deficiency |
| PKR | Pyruvate kinase isoform R |
| PO | Oral |
| PPSV23 | Pneumococcal polysaccharide |
| PR | The portion of the ECG wave from the beginning of the P wave to the beginning of the QRS complex |
| PT | Preferred term |
| q12h | Every 12 hours |
| q24h | Every 24 hours |
| QD | Once-daily |
| QRS | QRS interval on an electrocardiogram |
| QTc | Heart-rate corrected QT interval |

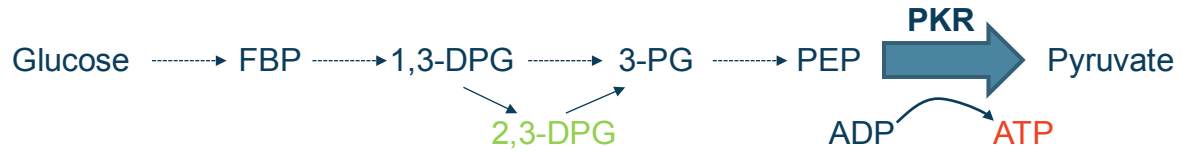
| Abbreviation or Specialist Term | Explanation |
|---|---|
| QTcB | Corrected QT interval - Bazett correction formula |
| QTcF | Corrected QT interval - Fridericia's method |
| RBC | Red blood cell (count) |
| RDW | Red cell distribution width |
| SAD | Single ascending dose |
| SAE | Serious adverse event |
| SAP | Statistical analysis plan |
| SD | Standard deviation |
| SOC | System organ class |
| SOP(s) | Standard Operating Procedure(s) |
| $t_{½}$ | Apparent terminal half-life |
| TIBC | Total iron-binding capacity |
| $T_{max}$ | Time to maximum concentration |
| TEAE | Treatment-emergent adverse event |
| ULN | Upper limit of normal |
| VS | Vital signs |
| $V_{ss}$ | Volume of distribution at steady-state |
| Vz/F | Mean apparent volume of distribution |
| WBC | White blood cell |
| WMA | World Medical Association |
| WOCBP | Women of childbearing potential |
| WT | Wild type |

# 5. INTRODUCTION

## 5.1. Pyruvate Kinase Deficiency

Pyruvate kinase deficiency (PK deficiency) is a glycolytic enzymopathy that results in non-spherocytic hemolytic anemia with a variable clinical presentation, ranging from mild or fully compensated forms to life-threatening neonatal anemia and life-long chronic hemolytic anemia associated with severe, debilitating co-morbidities. PK deficiency is caused by mutations in the PKLR gene, which results in defective pyruvate kinase isoform R (PKR) in the red blood cell (RBC). As shown in the diagram below, PKR catalyzes the final and irreversible step in glycolysis, converting phosphoenolpyruvate (PEP) to pyruvate, and formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP). Mature RBCs rely almost exclusively on the process of glycolysis to generate the energy carrier molecule ATP. PKR is thus a key enzyme for maintaining energy homeostasis in erythrocytes, and it has been proposed that ATP levels are critical for optimally maintaining RBC membrane integrity (van Wijk and van Solinge 2005).

**Figure 2: Red Cell Glycolysis**




Abbreviations: 1,3 DPG = 1,3 diphosphoglycerate; 2,3 DPG = 2,3 diphosphoglycerate; 3-PG = 3-phosphoglycerate; ADP = adenosine diphosphate; ATP = adenosine triphosphate; FBP = fructose 1,6-bisphosphate; PEP = phosphoenolpyruvate; PKR = red blood cell-specific form of pyruvate kinase
Note: Not all steps in glycolysis are shown.

The majority of mutations in PKR that have been described have a deleterious effect on PKR catalytic activity, protein stability, and/or protein expression. PK deficiency is associated with reduced RBC survival as well as impaired red cell maturation. Mature erythrocytes lack mitochondria relying predominantly on glycolysis to generate ATP. It is hypothesized that insufficient energy production promotes erythrocyte hemolysis due to impaired maintenance of cellular membrane homeostasis.

PK deficiency is an autosomal recessive disease, in which both homozygotes and compound heterozygotes develop hemolytic anemia. More than 200 different mutations in the PKLR gene have been identified in patients with PK deficiency to date, and the majority of these mutations are single nucleotide missense mutations (Zanella, et al. 2005). The prevalence of PK deficiency, based on the most common pyruvate kinase mutation in Southern Europe, is estimated at 51 cases per million in the Caucasian population (Beutler and Gelbart 2000).

Some patients with PK deficiency have a clinically severe disease course that can present as a severe hemolytic anemia in early infancy, requiring immediate care which typically includes blood transfusion support (Christensen, et al. 2011; Ghidini, et al. 1991; Hennekam, et al. 1990). Infants with PK deficiency may have erythroblastosis fetalis with hepatosplenomegaly, extramedullary hematopoiesis, and severe indirect hyperbilirubinemia, which is associated with a high risk of kernicterus. Hyperbilirubinemia associated with the hemolytic state often requires

supportive treatment with intensive phototherapy. For other infants, the hyperbilirubinemia caused by hemolysis can require exchange transfusion, as well as supportive transfusion to keep their hematocrit (HCT) levels >60%.

Adults with PK deficiency are reported to have life-long hemolysis and the subsequent associated co-morbidities including anemia and transfusion dependence. Other co-morbidities include frequent miscarriages, aplastic crises, as well as symptoms associated with an acute on chronic hemolytic anemia (Rider, et al. 2011). Since unconjugated bilirubin is often chronically elevated, pigmented gallstones are common in children and adults. Severe and sometimes life-threatening iron deposition occurs and is typically progressive.

The current therapeutic options for patients with PK deficiency are supportive. Most require life-long treatment, including blood transfusions at a frequency depending on the disease state. Long-term surveillance for systemic iron overload, even in transfusion-independent patients, is standard as is the use of chelation therapy. Case reports of cure by allogeneic bone marrow transplant have been published (Tanphaichitr, et al. 2000) but have been infrequently performed. Splenectomy, while not curative, can reduce the need for supportive blood transfusions and decrease bilirubin levels in some patients. However, this intervention is associated with significant morbidity including infection risk, and possible increased risk of thrombosis.

## 5.2. AG-348

AG-348 is an orally available, allosteric activator of PKR. It is hypothesized that drug intervention with AG-348 restores glycolytic pathway activity and normalizes red cell metabolism *in vivo*. Biochemical experiments demonstrate that AG-348 is a potent, pan-activator of many PKR alleles associated with PK deficiency. PK deficiency red cells and their progenitors are characterized by changes in metabolism associated with defective glycolysis, including a build-up of PEP and 2,3-diphosphoglycerate (2,3-DPG), and lowered ATP levels. Treatment of PK deficiency patient red cells *ex vivo* with AG-348 results in increased ATP levels, and reductions in PEP and 2,3-DPG, consistent with pharmacological activation of PKR enzyme activity. *In vitro* studies against wild type (WT) PKR enzymes and *ex vivo* studies in blood from mice, monkeys and humans indicate that AG-348 activates WT PKR with a similar potency and efficacy as it does with the mutant versions. *In vivo* studies in mice confirm the potency of AG-348 in increasing WT PKR enzyme activity and modulating the levels of downstream markers such as ATP and 2,3-DPG. This therapeutic approach may be an effective way to correct the underlying pathology of PK deficiency and, importantly, provide clinical benefit to patients.

### 5.2.1. Summary of Nonclinical Data

A series of exploratory pharmacology studies were conducted to characterize the ability of AG-348 to activate WT PKR and anemia-associated PKR mutants (mPKR) *in vitro*, *ex vivo*, and *in vivo*. Additional details regarding nonclinical data can be found in the AG-348 Investigator's Brochure

#### 5.2.1.1. Pharmacology

AG-348 is a potent, broad-spectrum activator of PKR with low nM potency against both WT and mutant enzymes. AG-348 is also able to effectively stabilize mPKR enzymes that are

hypersensitive to thermal denaturation, suggesting that AG-348 may be able to increase PKR pathway activity by preventing destabilization and degradation of mPKR enzymes. Its major metabolite, AGI-8702, has low potency mixed activity against PKR and mutant isoforms and can increase the thermostability of some mPKR, albeit with significantly weaker potency than that of AG-348.

The effect of AG-348 and AGI-8702 on PKR activity and a number of downstream pathway markers was evaluated in human RBCs. AG-348 dose-response curves in human whole blood showed potent activation of PKR, while AGI-8702 is a weak activator of the PKR enzyme. In whole blood from mice treated with AG-348, $^{13}$C-glucose labeling through the PKR reaction was increased by 80% demonstrating AG-348-mediated activation of the WT PKR enzyme. AG-348 treatment of RBCs from healthy human donors potently increased levels of the PKR reaction product ATP consistent with activation of PKR.

The effects of AG-348 on PKR activity and RBC metabolism also were assessed in blood samples from patients with PK deficiency. AG-348 activated PKR and induced metabolic changes (increased ATP levels and decreased 2,3-DPG levels) consistent with increased glycolytic pathway activity in RBCs from PK deficiency patients with different mutations in the PKR enzyme.

Assessments of binding inhibition potential and enzymatic activity were evaluated in a panel of 89 receptors, ion channels, and enzymes. Although AG-348 is a histamine H3 antagonist/inverse agonist and an aromatase inhibitor, no effects of histamine H3 modulation have been observed in safety pharmacology or toxicology studies. Effects consistent with aromatase inhibition were seen in toxicology studies (Section 5.2.1.4).

#### 5.2.1.2. Safety Pharmacology

Safety pharmacology studies demonstrated that neither AG-348 nor AGI-8702 exhibited significant inhibition of the human ether-à-go-go related gene (hERG) current (concentration of drug that achieved half-maximal inhibition [$IC_{50}$] 29.4 µM and > 10 µM, respectively). Additionally, in the monkey cardiovascular (CV) study, no CV effects were seen at any dose level. No effects were seen in AG-348 Good Laboratory Practice (GLP)-compliant Irwin and respiratory safety pharmacology studies in Sprague-Dawley rats. AG-348 administered via oral gavage had a dose-dependent emetic activity in the ferret at ≥ 30 mg/kg.

#### 5.2.1.3. Pharmacokinetics

Absorption, distribution, metabolism, and excretion (ADME) studies of AG-348 were performed in Sprague-Dawley rats, beagle dogs, and cynomolgus monkeys. Both oral (PO) and intravenous (IV) routes were evaluated at doses ranging from 1 to 50 mg/kg. A dose of 200 mg (50 mg/kg) was administered PO in the food-effect evaluation in monkeys. The PK of AG-348 in animal species is characterized by rapid oral absorption, medium to high total body plasma clearance (CLp), and high volume of distribution at steady-state ($V_{ss}$) in rats, dogs, and monkeys.

AG-348 has a medium to long apparent terminal half-life ($t_{1/2}$) in the rat, dog, and monkey. Medium to high oral bioavailability (F) was observed for AG-348 in animals. A high-fat diet led to lower oral absorption of AG-348 in monkeys.

AG-348 showed low brain penetration in rats (brain/plasma ratio of 5.7% to 9.1%) based on area under the plasma concentration versus time curve from 0 to 24 hours ($AUC_{0\ 24hr}$) following single and repeated-dose administration, respectively. Metabolism appears to be the major elimination pathway for AG-348 with minimal biliary or urinary excretion.

Both *in vitro* and *in vivo*, the predominant metabolite was the N-dealkylated product, AGI-8702. There were no metabolites unique to human liver microsomes and no direct Phase 2 metabolites. The plasma metabolite profile of AG-348 did not change with repeat or ascending dosing, suggesting that AG-348 is not susceptible to metabolic switching with increasing or prolonged dose administration.

AG-348 is a weak direct inhibitor of cytochrome P450 (CYP) 2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 (testosterone 6β-hydroxylation). There was no direct inhibition of CYP1A2, CYP2B6, or CYP3A4/5 (midazolam 1′-hydroxylation) by AG-348. There was evidence of metabolism-dependent inhibition of CYP2C19 (largely reversible) and CYP3A4 (largely irreversible) by AG-348. AG-348 is considered a weak time-dependent CYP3A4 inhibitor. AGI-8702 is a weak direct inhibitor of CYP2C9 and CYP2C19. AG-348 has the potential to cause an induction-related drug-drug interaction (DDI) with sensitive CYP2B6 and CYP3A4 substrates.

The routes of metabolism for AG-348 are via multiple CYPs with CYP3A4 contributing > 70% of the total. CYP1A2, CYP2C9, and CYP2C8 contribute approximately 6%, 10%, and 7% to the remaining metabolism of AG-348; other isoforms contribute < 4% each.

AG-348 is a substrate for P-glycoprotein (P-gp), but not breast cancer resistance protein (BCRP). AG-348 is an inhibitor of P-gp (91% and 99% inhibition at 41 and 411 μM, respectively), but does not inhibit BCRP. AGI-8702 is not an inhibitor of P-gp or BCRP under the concentrations tested (5 and 100 μM).

#### 5.2.1.4. Toxicology

In rats, the no-observed-effect level/no-observed-adverse-effect level (NOEL/NOAEL), determined as 2000 mg/kg, was associated with area under the plasma concentration versus time curve from 0 to 12 hours ($AUC_{0\text{-}12hr}$) values 223- to 526-fold the projected human efficacious $AUC_{0\text{-}12hr}$ value. In dogs, clinical observations consistent with anaphylactoid reactions were seen, and the maximum tolerated dose (MTD) was 62.5 mg/kg, which was associated with an $AUC_{0\text{-}12hr}$ value 5.7-fold the projected efficacious $AUC_{0\text{-}12hr}$ value. The NOEL/NOAEL in dogs was 10 mg/kg, which was associated with an $AUC_{0\text{-}12hr}$ value 0.8-fold the projected efficacious $AUC_{0\text{-}12hr}$ value. In monkeys, the NOAEL was 1000 mg/kg, with only non-adverse emesis and body weight loss seen; this dose was associated with an $AUC_{0\text{-}12hr}$ value 70-fold the projected efficacious $AUC_{0\ 12hr}$ value. Based on the results of single-dose studies, the rat and the monkey were chosen as the most appropriate species for further evaluation in toxicology studies.

Dose-limiting toxicity (DLT) in cynomolgus monkeys was defined in non-GLP 5-day and 14-day repeat-dose studies as emesis, inappetence, and weight loss. These toxicities became dose limiting at $AUC_{0\text{-}12hr}$ values 27- to 34-fold the projected efficacious $AUC_{0\text{-}12hr}$ value, and precluded meaningful evaluation of other toxicities at this exposure level. Potential other effects at this exposure level were observed in a number hematology and serum chemistry parameters as well as in lymphoid organs. Additionally, minimal potential effects in kidneys (renal

tubulointerstitial nephritis) and heart (myocardial degeneration), which could not be differentiated from spontaneous background lesions, were seen when monkeys were exposed to AG-348 $AUC_{0\text{-}12hr}$ values ≥ 27- to 34-fold the projected human efficacious $AUC_{0\text{-}12hr}$ value.

In GLP-compliant 28-day monkey study, the dose of 150 mg/kg/day (75 mg/kg/dose twice daily [BID]) was the NOAEL. Effects were limited to increased liver weights without serum chemistry or microscopic correlate. At this dosage level, the Day 27 $AUC_{0\text{-}12hr}$ values were 8.9- and 8.5-fold the projected efficacious $AUC_{0\text{-}12hr}$ value in males and females respectively. In the same study, the low dosage of 20 mg/kg/day (10 mg/kg/dose) resulted in $AUC_{0\text{-}12hr}$ values that approximated the efficacious $AUC_{0\text{-}12hr}$ value, and there were no test article-related effects seen. The next highest dose of 50 mg/kg/day (25 mg/kg/dose) was the NOEL and was associated with $AUC_{0\text{-}12hr}$ values 3.1- and 2.6-fold the projected efficacious $AUC_{0\text{-}12hr}$ value in males and females respectively.

In the GLP-compliant 28-day rat study, the NOAEL in males was the highest dose tested, 600 mg/kg/day (300 mg/kg/dose), and the NOAEL in females was the lowest dose tested, 20 mg/kg/day (10 mg/kg/dose). At the 600 mg/kg/day dosage level in males, AG-348-related findings were limited to mild effects on hematology, serum chemistry, and urinalysis parameters, and microscopic findings in the adrenal gland (minimal to mild vacuolation of the adrenal zona glomerulosa and decreased thickness of the zona fasciculate), liver (minimal to mild hepatocellular hypertrophy), kidney (minimal tubular vacuolation), pancreas (minimal to moderate decreased zymogen granules), heart (minimal myocardial vacuolation), and prostate (minimal to mild decreased secretion). All findings were fully reversible over the 14-day recovery period with the exception of decreased serum glucose levels and decreased prostate secretion. In females, the highest dosage tested was 200 mg/kg/day (100 mg/kg/dose); adverse effects observed were similar to those observed in the 600 mg/kg/day males, with the exception that in females, fewer effects in hematology and serum chemistry parameters were seen, and also in females, adverse effects in the reproductive organs consistent with aromatase inhibition were observed.

In the 13-week repeat-dose rat study, adverse effects in males were identified in the testes and consisted of seminiferous tubular degeneration, spermatid retention, and Leydig cell hypertrophy. These effects were defined as adverse at dose levels at which they are expected to impair fertility. These effects were either not present or present with incidence and severity similar to that of the vehicle group in lower dose levels. Adverse effects in females included uterine atrophy and increased folding of the luminal surface; these effects were defined as adverse at the dose level at which they are expected to impair fertility

In the 13-week repeat-dose study in monkeys, no adverse effects were identified, and no new effects were identified when compared to the 4-week repeat-dose study. Similar to what occurred on the 4-week study, inappetence and emesis during the initial 1-2 weeks of dosing occurred, precluding evaluation of higher doses.

AG-348 inhibits human aromatase activity with an $IC_{50}$ of 2050 nM (based on human placental microsomes) and rat aromatase with an $IC_{50}$ of 493 nM (based on rat ovarian microsomes). In the GLP-compliant 28-day rat study, histologic effects consistent with aromatase inhibition were seen in the female reproductive tract at the mid- and high-dosage levels (100 and 200 mg/kg/day) and included incomplete corpora lutea; ovarian follicular cysts; ovarian cystic, luteinized follicles; uterine atrophy; vaginal mucification; and altered cyclicity. Although these findings

were minimal to mild and were fully reversible (over 14 days), they were considered adverse and the next lower dosage evaluated, 20 mg/kg/day (10 mg/kg/dose BID) was the NOAEL in females. The Day 27 $AUC_{0\text{-}12hr}$ value associated with this dosage level was 6.9-fold the projected human efficacious $AUC_{0\text{-}12hr}$ value. The potential for aromatase inhibition effects occurring in female rats at $AUC_{0\text{-}12hr}$ values > 6.9-fold and < 53-fold the projected efficacious $AUC_{0\text{-}12hr}$ value has been addressed in a 13-week rat study. In this study using doses between the NOAEL and LOAEL in the 28-day study, the NOAEL for histologic lesions of the uterus that may be associated with aromatase inhibition resulted in an $AUC_{0\text{-}12hr}$ that was 26-fold the projected efficacious value. Notably, due to the potency difference of AG-348 against rat versus human aromatase inhibition, there is potential for a 4-fold wider margin for aromatase inhibition in humans versus rats. AGI-8702 is not an aromatase inhibitor.

### 5.2.2. Summary of Clinical Data

To date, 72 healthy adult volunteers have been exposed to AG-348 in 2 clinical studies, a single ascending dose (SAD) study and a multiple ascending dose (MAD) study, with 31 of these subjects exposed to a single AG-348 dose under fasted conditions; 5 exposed to 2 doses of AG-348 under fasted and fed conditions; and 36 exposed to repeated doses of AG-348 for up to 14 days[1]. The following discussion of clinical data refers only to healthy adult volunteer subjects, as this is the first clinical trial in which patients with PK deficiency will be treated with AG-348.

#### 5.2.2.1. Pharmacokinetics

The PK of AG-348 showed low to moderate variability between subjects. After single doses of AG-348 from 30 to 2500 mg, AG-348 was rapidly absorbed as indicated by the short time to maximum plasma concentration ($T_{max}$). There appeared to be some lengthening of the period of absorption at higher doses as indicated by higher $T_{max}$ and a less than proportional increase in maximum concentration ($C_{max}$). Dose-normalized area under the curve (AUC) generally remained constant over the dose range studied, suggesting that AG-348 total exposure increased in a dose-proportional manner. The mean $t_{1/2}$ ranged from 17.8 to 20.4 hours when samples were collected through 72 hours and from 50.3 to 79.3 when samples were collected through 120 hours. However, this terminal elimination phase contributed little to overall exposure of AG-348, as indicated by the small difference between $AUC_{0\text{-}12}$ and area under the plasma concentration versus time curve from 0 to infinity ($AUC_{0\text{-}\infty}$), suggesting a shorter effective half-life of approximately 3 to 6 hours. AG-348 was extensively distributed (mean apparent volume of distribution [$V_z/F$] range of 271 to 1148 L) and had a moderate rate of clearance (geometric mean clearance [CL/F] range of 10.3 to 13.9 L/hr). The fraction of AG-348 excreted in urine ranged from 0.0145 to 0.0209 across the dose levels suggesting that renal excretion plays a minor role in the systemic elimination of AG-348.

The preliminary repeat-dose PK of AG-348 at doses ranging from 15 mg every 12 hours (q12h) to 700 mg q12h also showed an increase in AG-348 exposure in a dose-proportional manner after the first dose. AG-348 exposure observed after repeated dosing for 14 days at doses of

[1] At the time of this document, results from ongoing Study AG348-C-002 in healthy volunteers are blinded. Based on the randomization scheme, 36 subjects were assigned to AG-348 and 12 subjects were assigned to placebo.

120 mg every 24 hours (q24h) to 700 mg q12h were lower than observed on Day 1 after the first dose. The magnitude of the effect was greater at higher doses; lower doses of 15 mg q12h and 60 mg q12h did not show this effect. This observation may be related to the nonclinical finding that AG-348 is a potential inducer of human CYP3A4, an enzyme which biotransforms AG-348. It is possible that multiple doses of AG-348 leads to an increased rate of its own metabolism.

Evaluation of the effect of food on the PK of a single 700 mg dose of AG-348 in 5 subjects who were administered the drug fasting and then, after an appropriate wash-out period, re-administered the drug following ingestion of a standard US Food and Drug Administration (FDA) high fat meal, showed that food likely has a minimal effect on the PK of AG-348.

#### 5.2.2.2. Pharmacodynamics

After a single dose of AG-348, a decrease in the concentration of 2,3-DPG was observed at 3 hours post-dose, decreased in a dose-dependent manner to a minimum at 24-hour post-dose, and then returned to values similar to baseline by 72 to 120 hours post-dose. The mean decrease at 24 hours was approximately 300 µg/mL at the 700 through 2500 mg dose levels. Similar decreases were observed after the first dose of multiple doses and prior to doses in the midst of the MAD study. After the final dose of multiple doses, the concentration of 2,3-DPG returned to values similar to baseline between 72 and 120 hours post- dose.

After a single dose of AG-348, a minimal increase in the concentration of ATP was observed at 24 to 120 hours post-dose. In the MAD study, no increase was observed at 12 hours after the first dose; the concentration of ATP was increased on Day 8 to concentrations greater than in the single dose study, continued to trend upward on Day 11, and remained at a similar level through Day 14. The concentration of ATP remained elevated through 120 hours after the last dose on Day 14. The magnitude of the increase in ATP was similar across the dose range from 60 mg BID to 700 mg BID.

#### 5.2.2.3. Safety

Overall, AG-348 has been well-tolerated among healthy volunteers.

After a single AG-348 dose, treatment-emergent adverse events (TEAEs) reported by > 1 subject at any time on study (either under fasted or fed conditions) included headache (22%), nausea (14%), and contact dermatitis and vomiting (each 6%). After repeated dosing of AG-348 for 14 days, based on blinded data, TEAEs that occurred in > 2 subjects within any cohort and therefore must have been experienced by at least 1 AG-348-treated subject included dizziness, feeling hot, headache, hyperhidrosis, nausea, restlessness, and vomiting.

A dose-relationship was apparent with regard to the incidence of gastrointestinal events, primarily nausea and vomiting, with the incidence of such events increasing with increasing dose. Nausea and/or vomiting were observed only at doses ≥ 1400 mg in the single dose study and only at doses ≥ 700 BID in the MAD study. Nausea and vomiting were not observed at any dose ≤ 360 mg in either the single or multiple dose studies.

All but 1 TEAE reported to date has been mild or moderate (Grade 1 or 2) in intensity. The only Grade 3 TEAE was elevated liver transaminases (alanine aminotransferase [ALT]; aspartate aminotransferase [AST]) in a single subject in the MAD study treated with 700 mg of blinded study drug q12h BID. The treatment this subject received (AG-348 *versus* placebo) remains

blinded, and the events were considered to be study drug-related and led to study drug discontinuation, following which the elevated liver transaminases resolved. No AG-348-treated subject discontinued in the SAD study due to an adverse event (AE). Four subjects, at doses of 700 mg BID, have discontinued study drug in the MAD study due to at least 1 TEAE, with nausea and vomiting contributing to study drug discontinuation in 3 of these 4 subjects. The fourth subject discontinued for a cutaneous rash.

No deaths or other serious adverse events (SAEs) have been reported in any clinical study of AG-348. Furthermore, no DLTs have been declared after a single administration of AG-348 at doses of 30 mg to 2500 mg or after repeat administration of AG-348 for 14 days at daily doses of 120 mg to 1400 mg (although the event of Grade 3 elevated liver function tests described above in the MAD study may be assessed as DLT after the subject's treatment assignment is unblinded).

Due to preclinical observations pertaining to the potential for inhibition of the aromatase enzyme (see Section 5.2.1.4), the AG348-C-002 study included assessment of baseline and serial measures of free and total serum testosterone and serum estradiol and estrone. The data are still blinded, and therefore, no quantitative statistical analyses have yet been performed. Upon qualitative review of the serum hormone data, at least some subjects in the 120 mg BID cohort and higher dose cohorts demonstrate modest increases in androgenic hormones and decreases in estrogens, compatible with a potential signal of aromatase inhibition. Additional analyses will be performed after the study is completed and the data are locked and unblinded.

## 5.3. Study Rationale

Study AG348-C-003 is the first study that will be conducted in patients with PK deficiency. This study is primarily intended to evaluate the safety and tolerability and potential indicators of clinical activity of AG-348 administered for up to 24 weeks. This study will also evaluate the PK profile of AG-348 and its metabolite AGI-8702, the PD responses in ATP and 2,3-DPG following administration of AG-348, and the clinical activity of AG-348 in PK deficiency patients. Two previous double-blind, placebo-controlled clinical trials of AG-348 conducted in healthy adult male and female volunteers (AG348-C-001, a SAD study; and AG348-C-002, a MAD study) have established an acceptable safety and tolerability profile for AG-348 for up to 14 days of both once-daily (QD) and BID dosing at exposures that result in significant pharmacodynamic (PD) changes in whole blood levels of the glycolytic metabolites 2,3-DPG and ATP. Due to the potential for AG-348-mediated aromatase inhibition, dual-energy x-ray absorptiometry (DXA) scan (hip and spine) will be performed at Screening (if patient has not had prior DXA scan within 3 months of Day 1) to obtain T and Z scores. These data will serve as baseline measures of bone mineral density for all enrolling patients (see Section 7.1 for more details).

The target population of this study consists of adult males and females with a diagnosis of PK deficiency, who are anemic but non-transfusion dependent. Non-transfusion dependent patients are preferred for this study in order to reduce any potential confounding effect of transfusion therapies on evaluation of potential indicators of clinical activity and PD responses. The safety, tolerability, and PK/PD findings in this study will form the basis for subsequent clinical development of AG-348.

### 5.3.1. Summary of Overall Safety Management Plan

Measures to minimize the risks to patients enrolled in this study have been taken with respect to the following study design elements:

- The initial doses for Arms 1 and 2 have been selected on the basis of safety and tolerability observations already carefully made in two predecessor clinical trials in adult healthy male and female volunteers;
- The specified inclusion/exclusion criteria have been carefully considered to avoid enrollment of subjects for whom exposure to the study drug might pose a hazard;
- A designated Data Review Team (DRT) consisting of the overall study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer has been established to meet regularly at approximately 6-week intervals throughout the duration of the study to review the accumulating study data and will exercise options to suspend enrollment to one or both of the initial two study dose arms, discontinue enrollment to one or both of the initial two study dose arms, adjust the dose of patients in one or both of the initial two study arms, and/or implement one new study dose arm. If one new dosing arm is implemented, the dose selected will not exceed 360 mg BID, the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy volunteers. Group cohort stopping rules for terminating enrollment into an arm based on the severity (CTCAEv4.03 grade) and frequency of AEs are defined;
- Dose modification and stopping rules are defined for individual patients;
- Guidance for permitted, prohibited, and cautionary concomitant medications is specified based on the estimated potential for drug-drug interactions from hepatic cytochrome enzyme interactions with AG-348.
- Due to the potential for AG-348-mediated aromatase inhibition, bone mineral density will be monitored using DXA scans (hip and spine) at Baseline (if patient has not had prior DXA scan within 3 months of Day 1) and between Week 24 and Week 28.

# 6. TRIAL OBJECTIVES AND ENDPOINTS

## 6.1. Primary Objective

The primary objective of the study is to:

- Evaluate the safety and tolerability of up to 24 weeks of AG-348 administration in patients with PK deficiency.

## 6.2. Secondary Objectives

The secondary objectives of the study are to:

- Evaluate the pharmacokinetics (PK) of AG-348 and the metabolite AGI-8702.
- Evaluate the PD response of ATP and 2,3-DPG after administration of AG-348.
- Evaluate indicators of clinical activity of AG-348 in patients with PK deficiency, including changes in hemoglobin (Hb), HCT, reticulocyte count, haptoglobin (Hp), carboxyhemoglobin (COHb), lactate dehydrogenase (LDH), total and indirect bilirubin, erythropoietin (EPO), ferritin, and transferrin saturation (serum iron/iron binding capacity).

## 6.4. Study Measures and Endpoints

### 6.4.1. Safety Measures and Endpoints

Safety will be evaluated by:

- Monitoring of AEs, including determination of SAEs and AEs leading to discontinuation; safety laboratory parameters (hematology, chemistry, urinalysis, coagulation); physical examination findings; vital signs (VS); 12 lead electrocardiograms (ECGs); and DXA scans. Adverse events will be graded using Common Toxicity Criteria for Adverse Events (CTCAE), Version 4.03. Serum sex hormone levels (testosterone, estrone, and estradiol), bone turnover markers (serum N-terminal telopeptide [NTX] and serum C-terminal telopeptide [CTX]), total cholesterol, high-density lipoprotein-C (HDL-C), and triglycerides will be monitored for evidence of potential inhibition of aromatase by AG-348.

### 6.4.2. Clinical Activity Measures and Endpoints

- Monitoring of potential indicators of clinical activity will include evaluating changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation.

### 6.4.3. Pharmacokinetic and Pharmacodynamic Measures and Endpoints

The PK and PD profile of AG-348 will be evaluated by:

- Approximately the first 10 patients treated, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 15.1, Table 5. The remainder of treated patients will undergo limited PK sampling as detailed in Appendix 15.1, Table 6. Serial blood sampling for determination of concentration-time profiles of AG-348 and its metabolite AGI-8702 will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of AG-348 and AGI-8702 will be obtained. AG-348 and AGI-8702 will be analyzed using qualified assays to determine concentrations in plasma. Pharmacokinetic parameters on Day 1 and Day 15 will be computed using standard non-compartmental methods based on observed plasma AG-348 and AGI-8702 concentrations.
- Pharmacodynamic assessments will include 2,3-DPG, ATP (secondary objectives), [redacted] Approximately the first 10 patients treated will undergo extensive PD sampling as detailed in Appendix 15.1, Table 5. The remainder of treated patients will undergo limited PD sampling as detailed in Appendix 15.1, Table 6. Serial blood sampling for determination of levels of ATP and, 2,3-DPG will be conducted following the first dose and the morning Day15 dose, and additional trough levels of ATP and 2,3-DPG will be obtained. ATP and 2,3 DPG will be analyzed using qualified assays to determine concentrations in whole blood. PD parameters on Day 1 and Day 15 will be computed based on observed whole blood ATP and 2,3-DPG concentrations.

[redacted]

# 7. INVESTIGATIONAL PLAN

## 7.1. Overall Study Design

Study AG348-C-003 is a Phase 2, open-label, two-arm, multicenter, randomized, dose-ranging study during which adult patients with PK deficiency will receive multiple doses of AG-348 for up to 24 weeks. Patients with PK deficiency confirmed by red blood cell PK enzymatic assay performed at Screening will be eligible to participate in this study. At Week 25, patients who safely tolerate AG-348 and demonstrate clinical activity of AG-348 may be eligible to immediately roll over to a separate safety extension study for continued treatment. Patients who finish treatment at the end of 24 weeks or sooner will undergo follow-up assessment 4 weeks after the last dose of study drug. Patients with toxicity suspected to be related to study drug will continue follow-up until the AE resolves, is declared chronic by the Investigator, or the patient is lost to follow-up.

Initially, up to 25 patients will be randomized on an open-label, 1:1 basis to each of two BID doses of AG-348 (up to 50 patients; see Figure 3, Study Schema). The dose of Arm 1 is 300 mg of AG-348 administered orally q12h (BID). The dose of Arm 2 is 50 mg of AG-348 administered orally q12h (BID) for 12 weeks. Randomization will be stratified by *PKR* mutation in order to maintain balance as much as possible across the dose arms for the specific mutations expected to be most frequently enrolled. The *PKR* mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H) and all other mutations ("other"). Mutation status is defined by the presence of at least one of the indicated mutations; patients with more than one stratified mutation will be assigned based on Sponsor's discretion.

The doses for each arm have been selected from the forerunner AG348-C-001 SAD study and AG348-C-002 MAD studies in healthy adult volunteers to represent the range of doses/exposures that were safely tolerated and resulted in maximal or near maximal PD effects on 2,3-DPG and ATP.

Because PK deficiency is a rare disease with a limited eligible patient population and because the underlying pathophysiology and clinical phenotype of affected patients is heterogenous due to the wide variety of mutations in PKR that cause the disease, it is deemed important to focus closely on dose findings in this first-in-patient study. Therefore, in addition to initiating this study with 2 different doses of AG-348 administered q12h, a DRT will be established to review study data on a frequent basis and adapt the study design, dose and schedule of AG-348 as indicated.

The DRT will monitor safety on an on-going basis and meet at regular intervals (approximately every 6 weeks), or *ad hoc* as necessary, to review AEs, VS, clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and ECGs on enrolled patients. The DRT will also review available PK/PD data and indicators of clinical activity (e.g., changes from baseline in Hb). The DRT will be comprised of the study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer.

Approximately every 6 weeks, beginning 6 weeks after the first patient is doses or *ad* hoc as necessary, the DRT will review cumulative safety data, available PK/PD data, and clinical

activity data. Based on the DRT's recurring 6-week reviews, the DRT may exercise one or more of the following options:

- Continue treatment and enrollment in existing arms without change.
- Add 1 new dose arm (Arm 3) to enroll up to 25 patients at a dose to be determined; the dose for Arm 3 may be lower or higher than Arm 1 and Arm 2 doses, but will not exceed 360 mg q12h; and the dose regimen may be less frequent than q12h.
- Terminate or suspend enrollment to allow further review of clinical data in Arm 1 and/or Arm 2 (and/or potential Arm 3). Enrollment in an arm could be terminated or suspended to allow further review, for example, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity.
- Re-assign patient's doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the patients in the terminated arm will remain in their original arm, i.e., they will not count towards the enrollment quota of the arm whose dose and schedule is being adopted.
- Implement specific genotype restrictions to enrollment in one or more arms to ensure representation of patients with genotypes of greatest clinical relevance.

The DRT will perform these evaluations on a recurring 6-week basis. The data that the DRT will review to make these decisions is expected to include, but are not necessarily limited to, the following:

- *Safety Observations:* all AEs; VS, clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and ECGs;
- *PK and PD Observations:* including changes in 2,3-DPG and ATP;
- *Indicators of Clinical Activity:* including changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, EPO, total and indirect bilirubin, ferritin, and transferrin saturation.

If a third dose arm is implemented, the dose of AG-348 selected will not exceed 360 mg BID, as this was the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy volunteers. The PK/PD sampling schedule in a potential third dosing arm will be determined by Sponsor's discretion and may follow either the extensive or limited PK/PD sampling schedules as specified.

The DRT will monitor the safety data in an ongoing manner as described and may make a decision to terminate enrollment in an arm if unacceptable AEs are observed, PD activity is limited, and/or markers of clinical activity indicate no effect. Unacceptable AEs are defined as ≥ Grade 3 AEs (using National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAEv4.03]) that are assessed as at least possibly related to AG-348, with the exception of Grade 3 headache, hot flash/flushing, nausea, vomiting, and/or diarrhea that is transient in duration (< 24 hours) or able to be medically managed to ≤ Grade 2 within 24 hours. The DRT may also exercise discretion to terminate enrollment in an arm if it observes a frequency of Grade 2 AEs that would make it difficult to support long-term dosing.

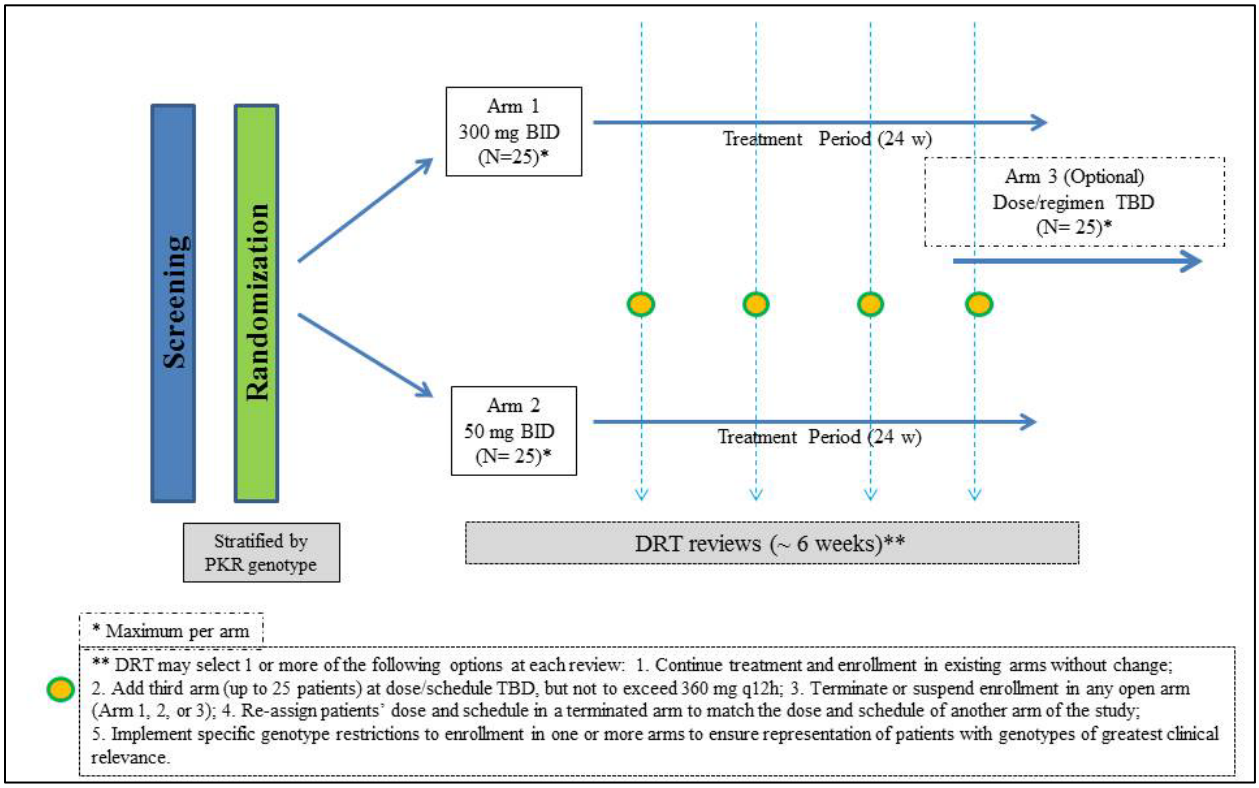
Due to the potential for AG-348-mediated aromatase inhibition, dual-energy x-ray absorptiometry (DXA) scan (hip and spine) will be performed at Screening (if patient has not

had prior DXA scan within 3 months of Day 1) to obtain T and Z scores. These data are intended to serve as a baseline measure of bone mineral density for all enrolling patients, and are deemed of particular importance for those who may enter the longer term safety extension study after completing the current study. All patients will have a second DXA scan in the interval between Weeks 24 and 28. The safety extension study will separately address additional follow-up DXA scanning.

As the number of enrolling arms changes in the study (for example, from 2 to 3), the randomization scheme will adjust to enable balanced randomization into each actively accruing arm. Randomization and stratification will cease in the event that only a single arm is left enrolling.

Depending on possible early termination of 1 or both of the initial 2 arms, or the addition of a third arm, the study could enroll up to a maximum of 75 patients.

**Figure 3: Study Schema**




Abbreviations: BID (q12h) = twice-daily (every 12 hours); DRT = data review team; PKR = pyruvate kinase red blood cell isoform; TBD = to be determined; w = weeks.

The Investigator will monitor all patients for safety and tolerability. Modification of an individual patient's dose of AG-348 will be based on AEs and observed changes in Hb levels as detailed in Section 9.7.1 and Section 9.7.2.

Screening assessments will occur within 28 days prior to the first dose of study treatment. During the Treatment period, patients will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12), and monthly through Week 24 (Weeks 16, 20, and 24). Patients who safely tolerate AG 348 and

demonstrate evidence of clinical activity of AG-348 through Week 24 may be eligible to immediately enter a separate extension study for continued treatment. For patients who finish treatment at the end of 24 weeks or sooner, or who elect not to enter the extension trial, study discharge will occur 4 weeks (Week 28 or earlier) following the last dose of study treatment at the final follow-up assessment.

Safety assessments will include monitoring of AEs, including determination of SAEs and AEs leading to discontinuation; safety laboratory parameters (e.g., hematology, serum chemistry, coagulation studies, and urinalysis); physical examination findings; VS; 12 lead ECGs, and DXA scans. Additional safety assessments will include monitoring of sex hormone levels (testosterone [total and free], estrone, and estradiol), and bone turnover markers (NTX and CTX). Follow-up assessments will be conducted on Day 197 (Week 28) and will include physical examination, weight, performance status, VS, 12-lead ECGs, laboratory evaluations (hematology, Hp, EPO levels, serum chemistry, coagulation studies, urinalysis; lipids, hormonal testing), AEs, and transfusion record.

Pharmacokinetic assessments will include serial blood sampling for PK profiles of AG-348 and its metabolite AGI-8702. Pharmacodynamic evaluations will include serial blood sampling for determination of levels of ATP and 2,3 DPG. Extensive PK/PD sampling will be conducted on the first approximately 10 patients total treated in Arms 1 and 2 (see Appendix 15.1, Table 5) while more limited PK/PD sampling will be conducted on additional patients treated if enrollment in Arm 1 or Arm 2 is expanded or if an alternate dosing arm is added (see Appendix 15.1, Table 6).

## 7.2. Justification of the Study Design

The primary and secondary objectives of this study are to evaluate the safety, tolerability, PK and PD, and indicators of clinical activity of AG-348 in patients with PK deficiency. The choice of dose and schedule of administration of AG -348 for Arms 1 and 2 was based on the highest safely tolerated dose (Arm 1: 300 mg BID) and the lowest dose with potentially relevant PD activity (Arm 2: 50 mg BID) from the forerunner AG348-C-002 MAD study in healthy volunteers. Decisions regarding continuing enrollment and treatment in these initial dose arms and/or implementation of an additional dose arm will be based on DRT review of safety, PK and PD data, and indicators of clinical activity collected from all patients treated in Arm 1 and Arm 2. This design was chosen to minimize risk to patients while allowing evaluation of safe and pharmacologically active dose levels of AG-348, and to allow the necessary flexibility to adjust dose and schedule should the safety, tolerability, PK, and/or PD be different in patients with PK deficiency compared with healthy volunteers.

Additional safety measures intended to minimize risk to patients include monitoring of AEs by the DRT and specified provisions for individual patient dose modification as needed for safety and (potentially) large increases in Hb level (Section 9.7.1 and Section 9.7.2). Measures intended to maximize the opportunity for patients with demonstrated safety and tolerability to continue to

derive benefit from any observed clinical activity of AG-348 include the option for continued treatment in a separate extension study.

A comprehensive series of safety evaluations, including laboratory parameters, physical examinations, VS, 12-lead ECGs, and monitoring for SAEs and AEs, will be conducted to evaluate the safety profile of AG-348 and to aid in the determination of the recommended dose for continued development.

The study includes serial blood sampling across at least two different doses of the study drug to assess its PK and PD profiles.

Consistent with the design of many Phase 2 studies, preliminary evaluation of the potential PD and clinical activity of AG-348 are secondary objectives of this study. The latter will include assessments of the PK/PD relationship between AG-348 and the biomarkers ATP and 2,3-DPG, [redacted]

## 7.3. Rationale for the Starting Dose and Dose Range

Prior to execution of this study, Agios conducted two clinical studies of AG-348 in healthy volunteers, including a SAD study (AG48-C-001) and a MAD (14 day q12h) study (AG348-C-002). Available details of these studies are discussed in the current Investigator's Brochure (IB). Between these two studies, 72 healthy human subjects have been dosed with AG-348. *In vitro* investigations, also reported in the IB, had previously demonstrated that AG-348 increased the activity of wild-type PKR approximately to the same extent as it did a series a recombinant mPKRs. Therefore it was deemed reasonable to study the safety, tolerability, PK, and PD of AG-348 in healthy subjects in a controlled Phase 1 setting as a more efficient means of obtaining information than in the actual rare disease population of patients with PK deficiency.

The MAD study demonstrated that the exposures produced by AG-348 doses from 60 mg q12h to 360 mg q12h (including 120 mg q24h) resulted in maximal changes from baseline for the PD markers 2,3-DPG (reduction from baseline) and ATP (increase from baseline). The exposures resulting from doses less than 60 mg q12h were of lesser magnitude and the exposures resulting from doses greater than 360 mg q12h were of no greater magnitude than the aforementioned range. Therefore the starting doses for this first dose ranging study in patients with PK deficiency were selected to be 300 mg q12h (Arm 1) and 50 mg q12h (Arm 2). These doses were demonstrated to be safe and tolerable in the healthy volunteer studies. The availability of ATP is proposed as being critical for optimally maintaining RBC membrane integrity (see Section 5.1). The dose ranges from 50 mg q12h to 300 mg q12h may result in clinically effective modulation of PKR in PK deficiency patients if the mutated enzyme is responsive to AG-348 in a similar manner to the wild-type enzyme in healthy subjects. However, there are many different mutations in PKR that result in PK deficiency, and these mutations produce variable effects on the enzyme in terms of catalytic activity and thermal stability. It is not known if different mutations will respond clinically in a similar manner to the same exposure to AG-348. Therefore, it is prudent to study the range of safe and pharmacodynamically relevant doses as specified in this study, and to allow flexibility for the DRT to analyze the evolving study data to adapt the dose and schedule of administration of AG-348 to produce the optimal combination of safety, tolerability, and PD, and, potentially, clinical response.

## 7.4. Criteria for Study Termination

This study may be prematurely terminated if, in the opinion of the Sponsor, there is sufficient reasonable cause. In the event of such action, written notification documenting the reason for study termination will be provided to each Investigator.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to patients;
- Insufficient adherence to protocol requirements;
- Plans to modify, suspend, or discontinue the development of the study drug;
- Other administrative reasons.

Should the study be closed prematurely, all study materials must be returned to the Sponsor or the Sponsor's designee.

# 8. STUDY POPULATION

## 8.1. Number of Patients

Up to approximately 75 patients may be enrolled in this study.

## 8.2. Inclusion Criteria

For entry into the study, patients must meet all of the following criteria during the Screening or other specified period:

1. Signed written informed consent obtained prior to performing any study procedure, including screening procedures.
2. Male or female, aged 18 years and older.
3. Known medical history of PK deficiency.
4. All patients must have documented clinical laboratory confirmation of PK deficiency by RBC pyruvate kinase enzymatic assay performed at Screening by a designated central laboratory. Patients with prior documentation of PK deficiency by RBC enzymatic assay will have a reconfirmation of this result during Screening as a condition of enrollment.
    a. In the event that a patient's screening pyruvate kinase enzymatic assay is negative (i.e., shows normal pyruvate kinase activity), the patient will be eligible for enrollment if the genotyping shows a mutant genotype that has been previously documented in the literature to be associated with pyruvate kinase deficiency. If the genotyping shows a previously undescribed mutation in the PKR gene, then the eligibility for enrollment will be determined on a case-by-case basis by the Coordinating Investigator and Medical Monitor in discussion with the Investigator. If no mutation is defined, then the patient will not be eligible.
5. All patients must have genotypic characterization of the mutant PKR gene performed by a designated central laboratory at Screening, unless genotype is available from the patient's participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480).
6. All patients must have genotypic characterization of the UGT1A1 gene performed by a designated central laboratory to document whether they may have underlying Gilbert's Disease. Patients with Gilbert's Disease are eligible to enroll.
7. Males must have Hb $\leq$ 12.0 g/dL; females must have Hb $\leq$ 11.0 g/dL.
8. All patients must be considered transfusion independent as defined by: no greater than 3 units of RBCs transfused in the 12-month period up to the first day of study dosing and no transfusions within 4 months of first day of study dosing. Patients who have received more transfusion support than described above will evaluated for eligibility on a case-by-case basis by the Medical Monitor.
9. Splenectomized patients:
    a. Must have undergone their procedure at least 6 months prior to Screening.

b. Must be current in their vaccinations for Pneumococcal Conjugate (PCV13), Pneumococcal Polysaccharide (PPSV23), Quadrivalent Meningococcal vaccine, and Haemophilus influenzae type b (Hib) as recommended by Centers for Disease Control and Prevention's (CDC) Advisory Committee on Immunization Practices (ACIP) or immunization advisory groups in Canada and the European Union (for patients enrolled in Canada and the EU). [http://www.cdc.gov/vaccines/schedules/downloads/adult/adult-schedule.pdf] [Any missing vaccinations may be administered during the screening period.]

10. Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 2.
11. Patients must be taking at least 1 mg of folic acid daily for at least 21 days prior to first dose and continued daily during study participation.
12. Adequate organ function, defined as:
    a. Serum AST and ALT ≤ 1.5 × upper limit of normal (ULN) (unless the increased AST is assessed by the Investigator as due to hemolysis).
    b. Normal or elevated levels of serum bilirubin. In patients with serum bilirubin > ULN, the elevation must be attributed to hemolysis with or without Gilbert's syndrome and must not be choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease.
    c. Serum creatinine ≤ 1.25 × ULN. If serum creatinine > 1.25 × ULN, then 24-hour measured or calculated (Cockcroft-Gault) glomerular filtration rate (GFR) ≥ 60 mL/min.
    d. Absolute neutrophil count (ANC) > 1.0 × 109/L.
    e. Platelet count ≥ 100 × 109/L.
    f. Activated partial thromboplastin time (aPTT) and international normalized ratio (INR) ≤ 1.25 × ULN, unless the patient is receiving therapeutic anticoagulants.
13. Women of childbearing potential (WOCBP) must agree to abstain from sexual intercourse or to use an acceptable/effective method of contraception (i.e., condom plus spermicide, condom plus oral contraceptive, condom plus intrauterine device [IUD], condom plus diaphragm with spermicide) from as soon as feasible during the Screening period until 30 days following the last dose of AG-348.
    a. WOCBP include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, and/or bilateral oophorectomy) or is not post-menopausal. Post-menopausal is defined as:
        i. Amenorrhea ≥ 12 consecutive months without another cause, and a documented serum follicle stimulating hormone (FSH) level > 35 mIU/mL;
        ii. Amenorrhea≥ 12 consecutive months in women ≥ 62 years old (FSH testing is not required).

14. WOCBP must have a negative serum or urine pregnancy test within 72 hours before start of AG-348 dosing.
15. Women must not be breastfeeding.
16. Male patients, with the exception of those who have undergone vasectomy at least 6 months prior to Screening, must agree to abstain from sexual intercourse or, if sexually active, to use a condom with spermicide as contraception (regardless of their female partner's childbearing potential or their partner's use of their own contraception) from Day 1 of dosing until 30 days following the last dose of AG-348.

## 8.3. Exclusion Criteria

Patients who meet any of the following criteria at Screening or prior to dosing on Day 1 will not be enrolled in the study:

1. Hemoglobin level > 12.0 g/dL if male; Hb > 11.0 g/dL if female.
2. Additional diagnosis of any other congenital or acquired blood disorder, including glucose-6-phosphate-dehydrogenase (G6PD) deficiency, or any other hemolytic anemia process except for mild allo-immunization as a consequence of transfusion therapy.
3. Iron overload (hemosiderosis or concurrent hemochromatosis) sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac, hepatic, or pancreatic insufficiency.
4. Prior bone marrow or stem cell transplant.
5. Clinically symptomatic cholelithiasis or cholecystitis. (Prior cholecystectomy is not exclusionary. Patients with symptomatic cholelithiasis or cholecystitis may be rescreened once the disorder has been treated and clinical symptoms have resolved.)
6. Currently enrolled in another therapeutic clinical trial involving on-going therapy with any investigational or marketed product or placebo. Concurrent participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480) is permitted.
7. Exposure to any investigational drug, device, or procedure within 28 days prior to Screening.
8. Concurrent medical condition that could compromise participation in the study such as:
    a. Poorly controlled hypertension (defined as systolic blood pressure (BP) > 150 mm Hg or diastolic BP > 90 mm Hg) refractory to medical management.
    b. History of recent (within < 6 months from Screening date) congestive heart failure; myocardial infarction or unstable angina pectoris; or hemorrhagic, embolic, or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.
    c. Currently active infection requiring the use of parenteral anti-microbial agents or that is ≥ Grade 3 (CTCAEv4.03) within 6 months of first dose.
    d. A pattern or frequency of post-splenectomy sepsis that in the assessment of the Investigator could reasonably be expected to interfere with the ability of the patient to complete the 24 week study participation.

e. Positive test for hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV) antibody with signs of active Hepatitis B or C virus infection.

f. Positive test for human immunodeficiency virus (HIV) 1 or 2 antibody.

g. Diabetes mellitus judged to be in poor control by the Investigator or requiring > 3 anti-diabetic agents counting insulin; use of insulin *per se* is not exclusionary.

h. History of any primary malignancy with the exception of: curatively treated non-melanomatous skin cancer; curatively treated cervical or breast carcinoma *in situ*; or other primary tumor treated with curative intent and no known active disease present and no treatment administered during the last 3 years.

9. Undergone major surgery within 6 months of first dose.
10. Current or recent history of psychiatric disorder that in the opinion of the Investigator or Medical Monitor could compromise the ability of the patient to cooperate with study visits and procedures.
11. Use of any of the restricted list of products known to strongly inhibit CYP3A4 metabolism (Appendix 15.3, Table 7) within 5 days prior to Day 1 dosing; or to strongly induce CYP3A4 metabolism (Appendix 15.3, Table 8) within 28 days prior to Day 1 dosing; or to strongly inhibit P-gp transporter (Appendix 15.3, Table 9) within 5 days prior to Day 1 dosing; or digoxin within 5 days prior to Day 1 dosing.
12. Serum bilirubin > ULN attributable to factors other than hemolysis and/or Gilbert's syndrome.
13. Male patients with heart-rate corrected QT (Fridericia's correction factor) QTcF interval > 450 msec, or female patients with QTcF interval > 470 msec with the exception of patients with a left bundle branch block (LBBB). Medical Monitor approval needed in patients with a LBBB.
14. Cardiac dysrhythmias judged as clinically significant by the Investigator or requiring therapy with drugs that are primarily substrates of CYP3A4.
15. History of allergy to sulfonamides if characterized by acute hemolytic anemia, anaphylaxis, or rash of erythema multiforme type or Stevens-Johnson syndrome.
16. Any other medical or psychological condition deemed by the Investigator to be likely to interfere with a patient's ability to understand and sign informed consent; cooperate with study visits, tests, and procedures; or otherwise safely and reliably participate in the study.

## 8.4. Patient Identification and Registration

Patients who are candidates for enrollment into the study will be evaluated for eligibility by the Investigator to ensure that the inclusion and exclusion criteria (see Section 8.2 and Section 8.3, respectively) have been satisfied and that the patient is eligible for participation in this clinical study. The site will submit to the Sponsor an Eligibility form for each eligible patient and the Medical Monitor will confirm eligibility for all patients prior to receipt of the first dose of AG-348.

## 8.5. Patient Randomization

Patients who have been confirmed as eligible will be randomized in an equal ratio to a treatment arm (e.g., 1:1 or 1:1:1 depending on which arms are open). A randomization number will be assigned. The site will provide a request for randomization form (including the patient's confirmed genotype) to a central randomization center. The randomization will be stratified by *PKR* mutation in order to maintain balance across the dose arms for the specific mutations expected to be most frequently enrolled. The *PKR* mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H) and all other mutations ("other"). Since this is an open label study, randomization will not be blinded.

Please refer to the study manual for the randomization procedure.

## 8.6. Patient Withdrawal Criteria

Patients have the right to withdraw from the study at any time for any reason. Patients may withdraw or be withdrawn from study-related procedures and treatments under the following conditions:

- Withdrawal of consent;
- Experiences unacceptable toxicity;
- Development of an intercurrent medical condition that precludes further participation in the trial;
- Patient requires use of a prohibited concomitant medication (Section 9.11.2);
- Investigator decision;
- Protocol violation: non-adherence to protocol requirements;
- Pregnancy;
- Lost to follow-up.

Should a patient decide to withdraw, all efforts will be made to complete and report the protocol-defined study observations up to the time of the patient's withdrawal as completely as possible and to determine the reason for withdrawal.

In the event a patient is withdrawn from the study, the Medical Monitor must be informed. If there is a medical reason for withdrawal, the patient will remain under the supervision of the Investigator until satisfactory health is returned.

When a patient withdraws from the study, the primary reason for discontinuation must be recorded in the appropriate section of the electronic case report form (eCRF) and all efforts will be made to complete and report final study observations as thoroughly as possible.

All AEs should be followed until resolution or for a period of 30 days from the last dose of study drug, whichever is shorter. Patients with toxicity suspected to be related to study drug will continue follow-up until the AE resolves, is declared chronic by the Investigator, or the patient is lost to follow-up.

## 8.7. Replacement of Patients

Patients who drop out of the study prior to completing the first 12 weeks of assigned dosing for reasons other than AEs may be replaced at the Sponsor's discretion.

# 9. STUDY TREATMENT

## 9.1. Description of Study Drug

AG-348 sulfate hydrate capsules will be provided as 25 mg or 100 mg (free-base equivalent) of AG-348 sulfate hydrate without excipients in Swedish orange (25 mg) or white opaque (100 mg) gelatin capsules (size 2 capsules for all dose strengths).

All study drugs are for investigational use only and are to be used only within the context of this study. All study drug products will be supplied by the Sponsor. Please see the Investigator's Brochure for further details regarding study drug.

## 9.2. Study Drug Packaging and Labeling

AG-348 sulfate hydrate capsules are packaged in white, high-density polyethylene (HDPE) induction sealed bottles with a child-resistant screw cap.

Packaging and labeling will be prepared to meet all regulatory requirements.

## 9.3. Study Drug Storage

AG-348 sulfate hydrate drug capsules must be stored at room temperature of 15 to 30°C (59 - 86°F).

All study drug products must be stored in a secure, limited-access location and may be dispensed only by the Investigator or by a member of the staff specifically authorized by the Investigator.

## 9.4. Method of Assigning Patients to Treatment

Up to a maximum of 25 patients will be randomized to any one of the dosing arms in this study. Randomization will be balanced 1:1 or 1:1:1, depending on whether 2 or potentially 3 arms are open, and will be stratified by *PKR* mutation (see Section 8.5). The dose and schedule of AG-348 each patient receives will be dependent upon which dose arm is open for enrollment when the patient qualifies for and is randomized into the study.

## 9.5. Blinding

This is an open-label study; no blinding methods will be used.

## 9.6. Study Drug Preparation and Administration

For the initial two treatment arms, (Arm 1 and Arm 2), AG-348 will be administered orally BID (approximately every 12 hours with a minimum of 10 hours between doses) over a 24 week treatment period. Starting with Day 1, dosing will be continuous; there will be no rest periods. Patients who do not meet any of the treatment withdrawal criteria (see Section 8.5) may continue treatment for the entire 24-week treatment period.

Patients will be dispensed the appropriate number of Sponsor-packaged, labeled bottles to allow for 28 days of dosing until the next scheduled visit.

Patients will be given a dosing diary to be used for each 28-day dosing period. They should record relevant information regarding their study drug in the diary (e.g., confirmation that each daily dose was taken, reasons for missed doses).

Treatment compliance will be assessed based on return of unused drug and the dosing diary (see Section 9.9).

Patients should be instructed to take their daily dose at approximately the same times each day except for dosing on in-clinic visiting days.

Patients who undergo extensive PK/PD sampling (see Appendix 15.1, Table 5) should be instructed from Week 3 on to bring the AM dose with them for in-clinic visits and to ingest the dose following PK/PD blood draws.

Patients receiving limited PK/PD sampling (see Appendix 15.1, Table 6) should be instructed to bring the AM dose with them for all in-clinic visits and to take the AM dose following PK/PD blood draws.

Patients receiving extensive PK/PD sampling on Day 1 and 15 will also have limited PKPD on other visit days. As a general rule, regardless of extensive or limited schedule, patients will bring in the AM dose for all visits and take this dose following PK/PD blood draws.

A minimum of 10 hours between the AM and PM dose will be required on those dosing days. Each dose should be taken with a glass of water and consumed over as short a time as possible. AG-348 may be taken with or without food. Patients should be instructed to swallow capsules whole and to not chew the capsules. For patients who have difficulty swallowing tablet(s), the Medical Monitor should be contacted to discuss administration.

Patients will receive their first dose of AG -348 in the clinic on Day 1 and then may take the remaining doses on an outpatient basis.

## 9.7. Criteria for Dose Escalation, Dose Modification, or Discontinuation of Study Drug

No intra-patient dose escalations will be permitted in this study unless the DRT decides to re-assign patients' doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the patients in the terminated arm will remain in their originally assigned arm; i.e., they will not count towards the enrollment quota of the arm whose dose and schedule is being adopted. All dosing modifications, as outlined below, will be implemented following discussions with the Medical Monitor.

### 9.7.1. Dose Modification for Safety

The Investigator will monitor all patients for safety and tolerability. Modification of the patient's dose of AG-348 will be based on AEs and observed changes in Hb levels (see Section 9.7.2).

**Table 1: Dose Modification for Adverse Events Deemed at Least Possibly Related to AG-348**

| Adverse Events(s) | AG-348 Dose Adjustment |
|---|---|
| Grade 1 | None required. |
| Grade 2 | None required; Investigator and Medical Monitor judgment to manage as for Grade 3. |
| Grade 3 | Suspend dosing;<br>If event resolves to Grade 1 or baseline within approximately 14 days of suspension, resume dosing with 1 dose level reduction (see Table 2 below).<br>If event does not resolve to Grade 1 or baseline within approximately 14 days of suspension, permanently discontinue dosing, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor. |
| Grade 4 | Permanently discontinue dosing, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor. |

Dose modifications for Grade 2 and 3 AEs should be maintained long enough for the Investigator to be confident that the reduced dose is being well tolerated before considering a request of the Medical Monitor to permit subsequent re-escalation to the former dose level. Dosing for an individual patient will be discontinued permanently for Grade 3 and Grade 4 AEs that do not resolve to Grade 1 or baseline within approximately 14 days of suspension of dosing, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor. No patients may be re-escalated to their former dose level after a dose modification without discussion with the Medical Monitor.

It should be noted that if the initial dose of 300 mg q12h BID selected for Arm 1 demonstrates an unacceptable safety profile resulting in multiple subjects undergoing dose modifications, the DRT may exercise its option to re-assign these patients' dose and schedule to match the dose and schedule of another study arm (for example, Arm 2 of the study, or to match the dose and schedule of a [potential] Arm 3, if implemented).

### 9.7.2. Dose Modification for Increase in Hemoglobin Level

It is presently unknown to what magnitude, how rapidly, or even whether, AG-348 will result in increased Hb levels in patients with PK deficiency across the variety of potential *PKR* mutations that may be encountered during this study. Moreover, it is unknown whether any potential increases in Hb experienced by patients in this study may be a safety risk. As a conservative measure, this study incorporates guidance for dose modification based on potential large increases in Hb levels that exceed the midpoint of the typical normal range by gender. The Investigator will monitor all patients for changes in Hb levels and should adjust the dose of AG-348 as outlined in Table 2 per the following guidelines and with Medical Monitor approval.

- Males: If Hb > 15 g/dL and confirmed with a second test, suspend dosing until Hgb ≤ 13.5 g/dL; then resume dosing with a 1 dose level reduction.
- Females: If Hb > 13.5 g/dL and confirmed with a second test, suspend dosing until Hb ≤ 12.5 g/dL; then resume with a 1 dose level reduction.

- The treating Investigator will discuss with the Medical Monitor questions relating to dose modifications on an as needed basis.

**Table 2: Dose Reduction Table (by Dosing Arm)**

| Dose Group | Starting Dose | 1st Dose Reduction | 2nd Dose Reduction |
|---|---|---|---|
| Arm 1 | 300 mg BID | 200 mg BID | 100 mg BID |
| Arm 2 | 50 mg BID | 25 mg BID | TBD[1] |
| Potential Arm 3 | TBD | To approximately 50-66% of initial dose | To approximately 25-33% of initial dose |

[1] Dose to be determined by Medical Monitor.

### 9.7.3. Stopping Criteria

Dosing for an individual patient will be discontinued permanently for Grade 3 AEs that do not resolve to Grade 1 or baseline within approximately 14 days of suspension of dosing, and for Grade 4 AEs, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor (Section 9.7.1). Other reasons for treatment termination are provided in Section 8.5.

## 9.8. Duration of Patient Participation

The duration of treatment for all patients on this study will be up to 24 weeks. Patients who safely tolerate and demonstrate one or more indicators of clinical activity of AG-348 may be eligible to immediately roll over to a separate safety extension study for continued treatment.

## 9.9. Treatment Compliance

During in-clinic visits, doses of AG-348 will be ingested by the patient under the supervision of clinical facility personnel. For at-home dosing, patients will be given a dosing diary to be used for the duration of the 24-week treatment period. Patients should record relevant information regarding their study drug in the diary (e.g., confirmation that each daily dose was taken, reasons for missed doses) and return the diary at each study visit.

## 9.10. Study Drug Accountability

Accountability for the study drug at the clinical facility is the responsibility of the Investigator. The Investigator will ensure that the study drug is used only in accordance with this protocol. Where allowed, the Investigator may choose to assign drug accountability responsibilities to a pharmacist or other appropriate individual.

The Investigator or delegate will maintain accurate drug accountability records indicating the drug's delivery date to the site, inventory at the site, use by each patient, and return to Agios or its designee (or disposal of the drug, if approved by Agios). These records will adequately document that the patients were provided the doses as specified in the protocol and should reconcile all study drug received from Agios. Accountability records will include dates, quantities, batch/serial numbers, expiration dates (if applicable), and patient numbers. An

unblinded monitor will review drug accountability at the site on a schedule agreed to by the Sponsor.

Study drug must not be used for any purpose other than the present study.

All unused and used study drug will be retained at the site until it is inventoried by the Study Monitor after database lock has occurred. All used, unused or expired study drug will be returned to Agios or its designee or, if authorized, disposed of at the study site per the site's Standard Operating Procedures (SOPs) and documented. All material containing AG-348 will be treated and disposed of as hazardous waste in accordance with governing regulations.

## 9.11. Prior and Concomitant Medications and Treatments

### 9.11.1. Prior Medications and Procedures

All medications administered and procedures conducted within 28 days prior to the first day of study drug administration are to be recorded on the source documentation and included in the eCRF.

### 9.11.2. Prohibited Concomitant Therapy

All concomitant medications and procedures administered from 28 days before administration of study drug through the last Follow-up Visit (Week 28/Day 197) must be recorded in the appropriate section of the source documentation and eCRF along with dosage information, dates of administration, and reason for use.

The following are prohibited at all times during participation in this study:

- Investigational drugs must be discontinued 28 days prior to the first dose of study drug;
- Products known to strongly inhibit CYP3A4 metabolism (listed in Appendix 15.3, Table 7) must be discontinued within 5 days prior to Day 1 dosing;
- Products known to strongly induce CYP3A4 metabolism (listed in Appendix 15.3, Table 8) must be discontinued within 28 days prior to Day 1 dosing;
- Products known to strongly inhibit P-gp transporter (listed in Appendix 15.3, Table 9) must be discontinued within 5 days prior to Day 1 dosing;
- Digoxin must be discontinued within 5 days prior to Day 1 dosing;
- Hematopoietic stimulating agents (erythropoietins, granulocyte colony stimulating factors, thrombopoietins, etc) must be discontinued no less than 28 days prior to the first dose of study drug. [Folic acid 1 mg orally per day is required for all patients. B12 injections are permitted for patients with a prior diagnosis of B12 deficiency syndromes, but must be repleted to stability of the Hb and mean corpuscular volume (MCV) prior to enrollment in the study];
- Anabolic steroids, including testosterone preparations, administered for anemia must be discontinued no less than 28 days prior to the first dose of study drug;

- As the target population for this study consists of transfusion independent patients and transfusion of blood products could confound key endpoints of the study, blood transfusions of any type must be strictly avoided except in cases of compelling medical need. If medical circumstances permit, the Medical Monitor should be contacted for discussion before any transfusions are administered.
- Other drugs that displace unconjugated bilirubin from albumin.

**Potential Drug Interactions**

Strong inhibitors of CYP3A4 (listed in Appendix 15.3, Table 7) are not permitted for use with AG-348. Based on modeling of AG-348 metabolism and tolerability data from the Phase 1 MAD study, moderate inhibitors of CYP3A4 do not appear to pose a risk to patients dosed with AG-348.

In-vivo data from the Phase 1 MAD show AG-348 induces CYP3A4 and its own metabolism, presumably via CYP3A4. The use of strong CYP3A4 inducers in combination with AG-348 is expected to reduce AG-348's efficacy. Therefore, administration of strong CYP3A4 inducers (listed in Appendix 15.3, Table 8) is not permitted with AG-348.

Strong inhibitors of drug transport (listed in Appendix 15.3, Table 9) are not permitted for use with AG-348.

Digoxin is not permitted for use with AG-348.

Induction of CYP3A4 by AG-348 is expected to reduce the efficacy of certain sensitive co-medications. Co-administration of AG-348 with the drugs in Appendix 15.3, Table 10 should be done with caution, as their efficacy may be reduced.

Of note, women in the trial utilizing oral contraception must utilize barrier methods as per the Inclusion Criteria 14 (Section 8.2) while taking AG-348.

The expected patient co-medications deferoxamine, deferasirox, and oral penicillin are not expected to interact with AG-348.

### 9.11.3. Allowed Concomitant Therapy

Medications and treatments other than those specified above are permitted during the study. All intercurrent medical conditions will be treated at the discretion of the Investigator according to acceptable local standards of medical care. Subjects may receive analgesics, antiemetics, anti-infectives (including penicillins), and antipyretics as medically indicated and consistent with the guidance in the previous two sections. Patients may continue iron chelation therapy with deferoxamine and deferasirox. Patients must continue taking 1 mg of folic acid for the duration of the study.

All concomitant medications, including transfusions of blood products, procedures performed during the study, including those used to treat AEs, will be recorded on the eCRF.

### 9.11.4. Potential for Phototoxicity

AG-348 may cause sensitivity to direct and indirect sunlight. Patients should be warned to avoid direct sun exposure. When exposure to sunlight is anticipated for longer than 15 minutes, the

patient should be instructed to apply factor 30 or higher sunscreen to exposed areas and wear protective clothing and sunglasses.

### 9.11.5. Management of Nausea, Vomiting, and Diarrhea

As the primary objective of this study is to assess the safety and tolerability of up to 24 weeks of AG-348, routine use of prophylactic anti-emetic and antidiarrheal or other classes of medications is prohibited. However, if patients experience AEs relating to nausea, vomiting, or diarrhea these may be treated at the Investigator's clinical discretion with recommended medications as follows:

- Nausea/vomiting: allowed agents include standard clinical dosing with palonosetron (Aloxi), dolasetron (Anzemet), or promethazine (Phenergen). Promethazine is a substrate for CYP2B6, and it is presently unknown if the potential for 2B6 induction after AG-348 dosing could be sufficient to reduce the therapeutic effect of promethazine. Not recommended are aprepitant (Emend; CYP3A4 inhibitor), ondansetron (Zofran; CYP3A4 inducer), chlorpromazine (Thorazine; CYP3A4 inducer), prochlorperazine (Compazine; CYP3A4 substrate, QT prolongation has been reported), and granisetron (Kytril; CYP3A4 substrate, QT prolongation has been reported);
- Diarrhea: recommended management includes standard clinical dosing with Kaopectate or other non-absorbable anti-diarrheals, diphenoxylate/atropine (Lomotil), or loperamide (Imodium). Loperamide is the least preferred choice because it is both a substrate and inhibitor for CYP3A4, a substrate for CYP2B6, and a substrate for P-gp.
- For the use of any medications not specifically mentioned above the Investigator may confer with the Sponsor’s Medical Monitor.

### 9.11.6. Other Restrictions and Precautions

Patients should be advised to refrain from altering their normal exercise routine for the first 28 days of treatment with AG-348 as symptoms of anemia could theoretically temporarily worsen before the eventual potential beneficial effect of AG-348 on the Hb level becomes evident. The elevated blood levels of 2,3-DPG commonly seen in patients with PK deficiency may produce a right shift in the Hb-O2 dissociation curve. The effect of this is to enhance oxygen delivery at the tissue level, thus counteracting to some extent the physiologic consequences of the anemia. AG-348 was shown to produce rapid decreases (within the first 12 hours following a single dose) in 2,3-DPG in two prior clinical trials with healthy adult male and female volunteers. In patients with PK deficiency who have elevated 2,3-DPG levels, it is theoretically possible that decreases in 2,3-DPG that precede correction of the anemia could result in a temporary decrease of oxygen delivery at the tissue level resulting in clinical symptoms (e.g., increased fatigue).

# 10. STUDY ASSESSMENTS

## 10.1. Schedule of Assessments

The Schedules of Assessments for this study are provided in Appendix 15.1.

After obtaining written informed consent, patients will undergo screening evaluations. The Screening visit is to be conducted within approximately 28 days prior to first dose of study treatment.

During the Treatment period, patients will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12) and monthly through Week 24 (Weeks 16, 20, and 24). Patients who safely tolerate and demonstrate one or more indicators of clinical activity of AG-348 through Week 24 may be eligible to immediately enter a separate extension study for continued treatment upon agreement of the treating Investigator and the Medical Monitor. For patients who finish treatment, Study Discharge will occur 4 weeks (Week 28 or earlier) following the last dose of study treatment at the final follow-up assessment.

Whenever more than one assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (e.g., VS first, ECG, blood draw). The timing of these assessments should allow the PK blood draw to occur at the exact nominal time. The order of procedures may be revised with prior discussion between Sponsor and Site.

Minor adjustments to the timing, number of planned safety monitoring procedures (e.g., VS, ECG, blood draw), and PK/PD assessments may be made during the course of the study based on collected data to ensure appropriate safety monitoring and will not require a protocol amendment. These minor changes will require prior approval from the Sponsor's Medical Monitor (or Responsible Medical Officer) as well as appropriate documentation in the study records. The addition of new safety monitoring procedures or other assessments will require a protocol amendment.

## 10.2. Informed Consent and Confirmation of Eligibility

A complete description of the study is to be presented to each potential patient and a signed and dated informed consent is to be obtained before any study specific procedures are performed.

Patient's eligibility will be confirmed at Screening and within 24 hours prior to study treatment dose. If a patient is determined to be ineligible, the patient will be excluded from participation.

## 10.3. Demographic Data, Medical and Medication History

Patient demographic data, including gender, date of birth, age, race, and ethnicity, will be obtained at Screening. Collection of demographic data will be modified by country regulatory requirements, as appropriate.

Medication history, including all relevant prior medical history and current medical conditions, will be obtained at the Screening assessment and on Day -1; all concomitant medications

administered and procedures conducted within 28 days prior to Day 1 should be reported in the source documentation and eCRF. Investigators will be asked to provide information on the patient's history of any medical diagnoses (e.g., iron overload) and surgical procedures (e.g., splenectomy, cholecystectomy) pertaining to their diagnosis of PK deficiency and prior available complete blood counts (CBCs) over the preceding 6 months and transfusion history over the preceding 12 months prior to the date of signing informed consent.

## 10.4. PKR Enzymatic Assay and PKR Genotyping

Assessments for PKR enzymatic activity and PKR genotyping will be performed at Screening only for confirmation of study eligibility. PKR enzymatic assays will be conducted at [redacted]. PKR genotyping will be conducted at [redacted].

## 10.5. Safety Assessments

### 10.5.1. Physical Examination, Height, and Weight

A complete physical examination (genital and rectal examinations will be performed at the discretion of the Investigator) will be obtained at Screening, Baseline, Week 12, and the Follow up Visit (Week 28). For patients rolling over into extension study, the last physical examination will occur at Week 24. Limited focused physical examinations will be performed at all other visits. Any findings will be recorded on the eCRF. Height will be collected at Screening only.

### 10.5.2. Vital Signs

Vital signs, including systolic and diastolic BP, heart rate, respiratory rate, and temperature, will be obtained according to the according to the Schedule of Assessments (Appendix 15.1). Vital sign measurements will be repeated if clinically significant or machine/equipment errors occur. Out of range BP or heart rate measurements will be repeated at the Investigator's discretion. Any confirmed, clinically significant vital sign measurements will be recorded as AEs.

### 10.5.3. Electrocardiogram

A 12-lead ECG will be obtained according to the Schedule of Assessments (Appendix 15.1). The ECGs will be measured using an ECG machine that calculates the heart rate and measures PR, QRS, QT, QTcB (Bazett correction formula), and QTcF (Frederica's correction). Only QTcF (not QTcB) will be used for determination of eligibility.

The 12-lead ECGs should be obtained following 5 minutes of recumbency. The Screening ECG will be performed at least 7 days prior to Day 1 dosing. ECGs will be repeated if clinically significant abnormalities are observed, if artifacts are present, or if machine/equipment errors occur. Any confirmed, clinically significant ECG findings will be recorded as AEs.

### 10.5.4. DXA Scans

DXA scans (hip and spine) will be performed at Screening to obtain T and Z scores that will serve as a baseline measure of bone mineral density for all enrolling patients. A second DXA scan will be conducted in the interval between Week 24 and Week 28 as indicated in the Schedule of Assessments (Appendix 15.1).

### 10.5.5. Safety Laboratory Assessments

#### 10.5.5.1. Hematology, Serum Chemistry, Coagulation Studies, and Urinalysis

Laboratory values obtained prior to Screening and RBC antibodies obtained at Screening will be performed at a local laboratory. On-study clinical laboratory evaluations are to be performed by a central laboratory. If Investigators believe that it is clinically indicated to obtain safety laboratory results from their own local laboratories on the day of the subject's visit, and before the results are returned from the designated central laboratory, they are free to exercise their discretion to do so. Investigators should be aware that since the Hb level is a secondary endpoint of the study, it behooves them to minimize blood volumes drawn, and samples for the central lab must still be collected, since it will serve as the official lab result for this study.

Clinical laboratory evaluations are to be collected according to the Schedule of Assessments (Appendix 15.1). In addition, all clinically significant laboratory abnormalities noted on testing will be followed by repeat testing and further investigated according to the judgment of the Investigator. Please note that serum estradiol and free and total testosterone will be collected in the AM at 2 time points during Screening at least 2 days apart in addition to Baseline/Day 1 (total of 3 time points prior to Day 1 dosing).

The following safety laboratory parameters are to be determined:

| | |
|---|---|
| **Hematology**: | HCT, Hb, RBC count, absolute reticulocyte count, percent reticulocyte count, MCV, mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count with manual differential, ANC, and absolute lymphocyte count (ALC), and platelet count. G6PD and RBC antibody screen will be performed at Screening only |
| **Other** | EPO, Hp, COHb |
| **Serum Chemistry:** | alkaline phosphatase (ALP), sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide ($CO_2$), albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, LDH, ALT, AST, total bilirubin, and indirect bilirubin. |
| **Sex Hormones**: | testosterone (total and free), estrone, and estradiol. FSH will only be performed at Screening for female patients only for confirmation of post-menopausal status. |
| **Bone Turnover** | serum NTX and CTX. |
| **Lipids** | total cholesterol, HDL-C, triglycerides. |
| **Iron Panel** | iron (Fe), total iron-binding capacity (TIBC), percent saturation, ferritin. |
| **Coagulation Studies:** | fibrinogen, aPTT, INR. |

**Urinalysis**: color and appearance, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, pregnancy screen (dipstick), occult blood. Microscopic inspection of sediment will only be performed for cause or to investigate an abnormal dipstick finding per the Investigator's discretion.

#### 10.5.5.2. Screening Serology

A blood sample for serology, including HBsAg, HCV Ab screen, and HIV1 and HIV2 Ab, is to be collected from all patients at Screening.

### 10.5.6. Adverse Events

Each patient will be carefully monitored for the development of any AEs throughout the study from signing of the informed consent through all scheduled study follow-up visits, or withdrawal of consent, whichever occurs first. In addition, SAEs that are assessed as possibly or probably related to study treatment that occur > 30 days post-treatment also are to be reported.

AEs will be evaluated by the Investigator and recorded as described in the Schedule of Assessments. On dosing visits, all AEs (elicited and spontaneously reported) will be continuously evaluated by the Investigator and recorded. At any non-dosing day visit, AEs will be evaluated by the Investigator and recorded. Any AEs already documented at a previous assessment and designated as ongoing will be reviewed at subsequent visits or assessment time points as necessary. If these AEs have resolved, this will be documented.

All AEs will be graded using the CTCAE grading system (Appendix 15.2 ).

Complete details on AE monitoring are provided in Section 11.

## 10.6. Pharmacokinetic Assessments

### 10.6.1. Blood Sample Collection and Pharmacokinetic Measurements During Dose Escalation

The first approximately 10 patients treated, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 15.1, Table 5. The remainder of treated patients will undergo limited PK sampling as detailed in Appendix 15.1, Table 6. In-clinic visits on Day 8 and Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis; in these instances PK sampling would not be required.

The collection times for post-dose PK samples will start from the time that dosing is completed. (For example, a PK draw at 30 minutes will be collected 30 minutes after the last capsule has been ingested.) The completion time of each dose will be recorded.

Procedures for sample collection and processing will be provided in a separate study manual. The actual time point of each sample collection will be recorded.

Samples for PK and PD assessments may be retained for up to 2 years from collection.

## 10.7. Pharmacodynamic Assessments

The first approximately 10 patients treated, contingent on clinical site feasibility, will undergo extensive PD sampling for 2,3-DPG and ATP as detailed in Appendix 15.1, Table 5. The remainder of treated patients will undergo limited PD for 2,3-DPG and ATP sampling as detailed in Appendix 15.3, Table 6.

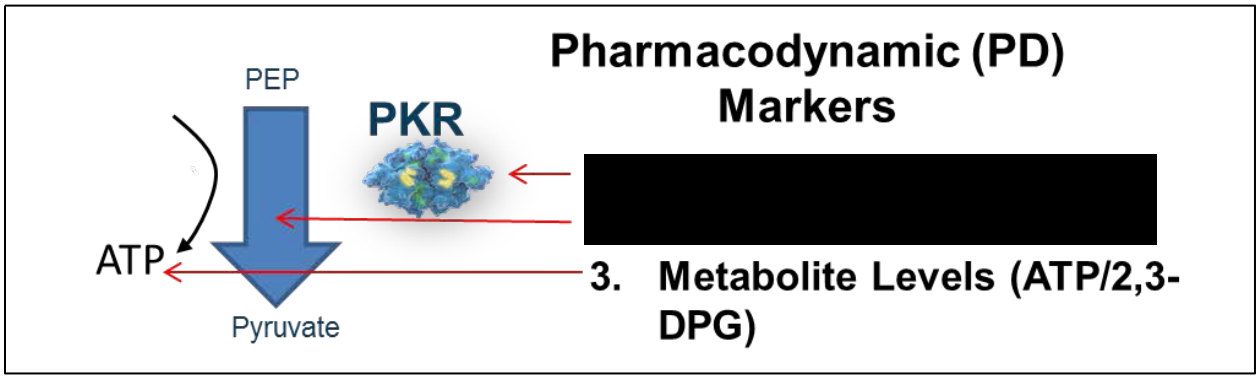
The collection times for post-dose PD samples will start from the time that dosing is completed. (For example, a PD draw at 30 minutes will be collected 30 minutes after the last capsule has been ingested.) The completion time of each dose will be recorded.

Procedures for sample collection and processing will be provided in a separate study manual. The actual time point of each sample collection will be recorded.

Pharmacodynamic assessments will include 2,3-DPG, ATP, [redacted] In-clinic visits on Day 8 and Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis; in these instances PK sampling would not be required.

Figure 4 provides a brief schematic outlining the PKR reaction and how each of these PD assessments fits into a complete mechanistic understanding of the action of AG-348.

**Figure 4: PKR Enzymatic Reaction**




The PKR enzyme catalyzes the PEP to pyruvate reaction, with concomitant formation of ATP.

17. Binding of AG-348 to the PKR tetramer can be assessed through an ex-vivo biochemical assay of cell lysates from AG-348 treated patients. Because WBCs contain a high level of pyruvate kinase from a non-PKR pyruvate kinase isoform, WBCs are first removed by filtration before the purified red cells are frozen. [redacted]
18. [redacted]

[redacted]

19. AG-348 target engagement [redacted] has been shown in preclinical models and healthy volunteer clinical studies to result in accumulation of ATP and depletion of the upstream metabolite 2,3-DPG. Therefore, levels of these metabolites will be measured by mass spectrometry in frozen whole blood samples.

The first approximately 10 patients treated, contingent on clinical site feasibility, will undergo extensive PD sampling as detailed in Appendix 15.1, Table 5. The remainder of treated patients will undergo limited PD sampling as detailed in Appendix 15.1, Table 6. The collection times for post-dose PD samples will start from the time that dosing is completed. (For example, a PD draw at 30 minutes will be collected 30 minutes after the last capsule has been ingested.) The completion time of each dose will be recorded.

Blood samples will be stored at the site and regularly transported at -80°C ±10 C to the bioanalytical laboratory for analysis. Procedures for sample collection and processing will be provided in a separate study manual.

## 10.8. Ordering of Blood Sample Collection

When more than 1 blood sample is collected at the same nominal time, the samples will be collected in the following order:

1. Safety laboratory assessments
2. PK
3. PD (2,3 DPG, ATP)

[redacted]

## 10.9. Sample Processing, Storage, and Shipment

Instructions for the processing, storage and shipment of all study samples for central analysis will be provided in a separate study manual.

# 11. ADVERSE EVENTS

Monitoring of AEs will be conducted throughout the study. Adverse events and SAEs will be recorded in the source documentation and eCRF from time of the signing informed consent through the Follow-up Visits. All AEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

All SAEs will be followed through 30 days after the last dose of study treatment or until the SAE has resolved. Any SAEs that are assessed as possibly or probably related to study treatment that occur > 30 days post-treatment also are to be reported.

## 11.1. Definition of Adverse Events

### 11.1.1. Adverse Event (AE)

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An AE can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

### 11.1.2. Suspected Adverse Reaction

A suspected adverse reaction is any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of expedited safety reporting, 'reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the AE.

### 11.1.3. Unexpected Adverse Event

An unexpected AE is one for which the nature or severity of the event is not consistent with the applicable product information, e.g., the Investigator's Brochure.

### 11.1.4. Serious Adverse Event

An AE or suspected adverse reaction is considered serious (SAE) if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death;
- Life-threatening. Life-threatening means that the patient was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form;
- In-patient hospitalization or prolongation of existing hospitalization. Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered AEs if the illness or disease existed before the patient was enrolled in the study, provided that it did not deteriorate in an unexpected manner during the study (e.g., surgery performed earlier than planned);

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- Congenital anomaly/birth defect;
- Important medical event. An important medical event is an event that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the patient or patient and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions for SAEs. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalization, or the development of drug dependency or drug abuse.

#### 11.1.4.1. Potential Severe Drug-Induced Liver Injury

The document entitled FDA Guidance for Industry, Drug-Induced Liver Injury: Premarketing Clinical Evaluation (FDA 2009) provides guidance on how the measurement of various laboratory parameters may be used to assess a given drug's potential to cause severe liver injury (i.e., irreversible liver failure that is fatal or requires liver transplantation). Such cases are suggested by the following three components:

1. The drug causes hepatocellular injury, generally shown by a higher incidence of 3-fold or greater elevations above the ULN of ALT or AST than the (non-hepatotoxic) control drug or placebo;
2. Among trial patients showing such aminotransferase elevations, often with aminotransferases much greater than 3×ULN, one or more also show elevation of serum total bilirubin to > 2×ULN, without initial findings of cholestasis (elevated serum elevated serum ALP);
3. No other reason can be found to explain the combination of increased aminotransferases and total bilirubin, such as viral hepatitis A, B, or C; pre-existing acute liver disease; or another drug capable of causing the observed injury.

Clinical safety laboratory results compatible with the definition of drug-induced liver injury (DILI) stated above must be repeated for confirmation as soon as possible, and if confirmed, will be scored as an unacceptable AE and reported to FDA as a serious unexpected AE.

## 11.2. Procedures for Reporting Adverse Events and Serious Adverse Events

Each patient must be carefully monitored for the development of any AEs. This information should be obtained in the form of non-leading questions (e.g., "How are you feeling?") and from signs and symptoms detected during each examination, observations of study personnel, and spontaneous reports from patients.

All AEs (serious and non-serious) spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures will be recorded in the source documentation and eCRF. Any clinically relevant deterioration in laboratory assessments or other clinical findings is considered an AE

and must be recorded in the appropriate source documentation and eCRF. Treatment-emergent abnormal clinical laboratory results should generally be reported as AEs if there are accompanying symptoms; if additional diagnostic evaluations or medical (including drug therapy) or surgical interventions are undertaken; if a change in study drug dosing or study drug discontinuation is required; or, if the laboratory result is considered clinically significant by the Investigator. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

All SAEs that occur during the course of the study must be promptly reported by the Investigator to [redacted] Global Safety and Pharmacovigilance (see below). Deaths and AEs assessed as life-threatening are to be reported immediately and SAEs that meet other criteria are to be reported within 24 hours from the point in time when the Investigator becomes aware of the SAE. All SAEs must be reported whether or not they are considered causally related to AG-348. Serious adverse event forms will be completed and the information collected will include subject number, a narrative description of the event, and an assessment by the Investigator as to the severity of the event and relatedness to study drug. Follow-up information on the SAE may be requested by the Sponsor or Medical Monitor.

**Contact Information:**

[redacted] **Safety Management**

SAE Hotline (United States): [redacted]
SAE Fax (United States): [redacted]

SAE Hotline (France): [redacted]
SAE Fax (France): [redacted]

SAE Hotline (Netherlands): [redacted]
SAE Fax (Netherlands): [redacted]

SAE Hotline (Italy): [redacted]
SAE Fax (Italy): [redacted]

SAE Hotline (United Kingdom): [redacted]
SAE Fax (United Kingdom): [redacted]

E-mail: [redacted]

If there are serious, unexpected adverse drug reactions associated with the use of AG-348, the Sponsor will notify the appropriate regulatory agency(ies) and all participating Investigators on an expedited basis. The local Institutional Review Board (IRB)/Independent Ethics Committee (IEC) will be promptly notified based on local regulations where required by the IRB/IEC of all serious, unexpected adverse drug reactions involving risk to human subjects.

All AEs, whether serious or not, will be described in the source documents in the database. All new events, as well as those that worsen in intensity or frequency relative to baseline, which occur after signing the informed consent through the final Follow-up Visit (Day 29 ± 3 days) must be recorded. Adverse events that are ongoing at the time of treatment discontinuation should be followed up to 30 days after the last dose of study treatment. All SAEs will be followed up to 30 days after the last dose of study treatment, or until the SAE has resolved.

Information to be reported in the description of each AE includes:

- A medical diagnosis of the event (if a medical diagnosis cannot be determined, a description of each sign or symptom characterizing the event should be recorded);
- The date of onset of the event;
- The date of resolution of the event;
- Whether the event is serious or not;
- Intensity of the event (see below for definitions);
- Relationship of the event to study treatment (see below for definitions);
- Action taken: none; change in the study drug administration (e.g., temporary interruption in dosing); drug treatment required; non-drug treatment required; hospitalization or prolongation of hospitalization required (complete SAE page); diagnostic procedure performed; patient discontinued from the study (complete End of Study visit);
- Outcome: patient recovered without sequelae; patient recovered with sequelae; event ongoing; patient died (notify the Medical Monitor immediately, and complete the SAE form).

**Intensity** of all AEs will be graded according to the NCI CTCAE Version 4.03 (Appendix 15.1).

**Relationship** to study drug administration will be determined by the Investigator according to the following criteria:

- **Not Related**: Exposure to the study treatment did not occur, or the occurrence of the AE is not reasonably related in time, or the AE is considered unlikely to be related to the study treatment.
- **Possibly Related**: The study treatment and the AE were reasonably related in time, and the AE could be explained equally well by causes other than exposure to the study treatment. An attribution of possibly related means that there are facts in evidence to suggest a possible relationship.
- **Probably Related**: The study treatment and the AE were reasonably related in time, and the AE was more likely explained by exposure to the study treatment than by other causes, or the study treatment was the most likely cause of the AE. An attribution of probably related means that there are facts in evidence to suggest a probable relationship.

For the purpose of safety analyses related to final database review, all AEs that are classified as possible or probable will be considered treatment-related AEs.

## 11.3. Pregnancy Reporting

Pregnancy is neither an AE nor an SAE, unless a complication relating to the pregnancy occurs (e.g., spontaneous abortion, which may qualify as an SAE). However, any pregnancy in a participating female patient or a female partner of a participating male patient that occurs during this study or within 30 days following the last dose of AG-348 must be reported to the Sponsor

or Medical Monitor within 30 days of being notified of the pregnancy. The Investigator must follow up and document the course and outcome of all pregnancies even if the patient was discontinued from the study or if the study has finished. The female patient or partner of a male patient should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus. Monitoring should continue until conclusion of the pregnancy.

All outcomes of pregnancy must be reported by the Investigator to the Sponsor or Sponsor's designee on a Pregnancy Outcome Report form within 30 days after he/she has gained knowledge of the delivery or elective abortion.

Any SAE that occurs during pregnancy must be recorded on the SAE report form (e.g., maternal serious complications, spontaneous or therapeutic abortion, ectopic pregnancy, stillbirth, neonatal death, congenital anomaly, or birth defect) and reported within 24 hours in accordance with the procedure for reporting SAEs.

All patients, male and female, must agree to use effective contraception during the entire study and for 30 days following the last dose of AG-348.

# 12. STATISTICAL METHODS

The primary objective of this Phase 2 study is to evaluate the safety and tolerability of up to 24 weeks of AG-348 in patients with PK deficiency. Therefore, analyses will be primarily descriptive in nature; no formal hypothesis testing will be conducted. All analyses will be conducted separately within each dose arm, or pooled where appropriate.

## 12.1. Sample Size Estimation

Due to the rare disease setting, the minimal sample size in each dose arm may be determined by feasibility. In order to evaluate the primary objective of safety and tolerability of AG-348 in Arm 1 and Arm 2, up to a maximum of 25 patients may be randomized onto each arm. The actual number of patients enrolled into Arms 1 and 2 will depend on the safety reviews and decisions made by the DRT. In addition, up to 25 additional patients may be enrolled to evaluate an additional dose arm (Arm 3; see Section 7.1). As for Arms 1 and 2, the actual enrollment in a potential Arm 3 will depend on the safety reviews and decisions made by the DRT. Therefore, up to approximately 75 total patients may be enrolled in this study across 2 to 3 dose arms.

Table 3 provides the probability within a dose arm of detecting 1 or more AEs with varying sample size and the true underlying AE rates.

**Table 3: Sample Size Estimation**

| Sample Size | True Underlying AE Rate | | |
|---|---|---|---|
| | **15%** | **10%** | **5%** |
| 5 | 56% | 41% | 23% |
| 10 | 80% | 65% | 40% |
| 15 | 91% | 79% | 54% |
| 25 | 98% | 93% | 72% |

## 12.2. Populations for Analysis

The following patient populations (i.e., analysis sets) will be evaluated and used for presentation of the data:

- Safety Analysis Set: All patients who are enrolled and receive any dose of study treatment. The Safety Analysis Set will be the primary set for the analysis of safety data. Patients will be classified according to treatment received, where treatment received is defined as the assigned treatment if it is received at least once, or as the first treatment received if assigned treatment is never received. Unless otherwise stated, the Safety Analysis Set will be the default analysis set for all data analyses.
- Pharmacokinetic (PK) Analysis Set: All patients in the Safety Analysis Set with sufficient plasma sample data to assess PK parameters. Results of the potential PD activity of AG-348 will also be based on the PK analysis set.

- Efficacy Analysis Set: All patients who are enrolled and achieve at least 50% compliance with their assigned dose intensity for at least 4 weeks of continuous dosing. Additional efficacy analyses may be performed on subsets of patients with other degrees of compliance with their assigned dose intensity and/or duration of dosing. The Efficacy Analysis Set will be the primary set for the analysis of preliminary clinical activity data. Patients will be classified according to assigned treatment.

[redacted] If such analyses are performed, they will be described in a separate PK Statistical Analysis Plan (SAP) and may be reported separately in a stand-alone report.

## 12.3. Procedures for Handling Missing, Unused, and Spurious Data

No imputation will be performed for missing data elements. When summarizing AE data, partial dates will be imputed as described in the SAP. Additional rules addressing the handling of missing data will be detailed in the SAP.

## 12.4. Interim Analysis

No formal statistical interim analysis will be conducted. Safety data will be reviewed on an ongoing basis by the DRT, who will meet to review safety, PK, PD, and preliminary clinical activity data at regular intervals (approximately every 6 weeks) throughout the duration of the study. The DRT's decisions to suspend, terminate, or open a potential third dosing arm, or re-assign patients' dosing in a terminated arm to match the dose and schedule of another arm of the study will be based on the totality of the data including, safety, PK, PD, and preliminary clinical activity (e.g., changes in Hb levels).

Additional interim reviews of data may be conducted to support decision making concerning the current clinical study, the sponsor's development programs in general, or in case of any safety concerns.

## 12.5. Statistical Methodology

### 12.5.1. General Methods

This study will be primarily descriptive in nature; therefore, there will be no formal hypothesis testing. Summaries will be produced for disposition, baseline disease characteristics and demographic data including genotype, safety measurements, PK, PD parameters and indicators of clinical activity. Data from each AG-348 dose group will be analyzed separately, and pooled across all dose groups where appropriate. All data will also be listed by individual patient.

Categorical variables will be summarized by frequency distributions (number and percentages) and continuous variables will be summarized by descriptive statistics (mean, standard deviation [SD], median, minimum, and maximum).

### 12.5.2. Disposition

A summary of the disposition of patients will be presented, including the number enrolled, the number treated, and the reasons for study discontinuation. Entry criteria and protocol deviations will be listed.

### 12.5.3. Exposure and Safety Analyses

Patients will receive multiple PO doses of AG-348 over a 24-week treatment period. The actual dose and duration in days of AG-348, as well as the dose intensity (computed as the ratio of actual dose received and actual duration) and the relative dose intensity (computed as the ratio of dose intensity and planned dose received/planned duration) will be listed and summarized using descriptive statistics by dose arm.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and the incidence of TEAEs (new or worsening from baseline) will be summarized by primary system organ class (SOC), preferred term (PT), CTCAE Version 4.03 severity, outcome, action taken with study drug, and relationship to the study drug by dose group. Separate summaries will be produced for all TEAEs, treatment-related AEs (those considered by the Investigator as at least possibly drug related), SAEs, discontinuations due to AEs, and AEs ≥ Grade 3 severity. Individual patient listings will be provided for deaths, SAEs, AEs leading to interruption and/or reduction of study drug dose, and AEs leading to discontinuation of treatment.

Analyses of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined as any AE with onset beginning at the day of first administration of study drug, throughout the treatment period until 30 days after the last dose of study drug, or any event that was present at baseline but worsened in intensity or was subsequently considered drug-related by the Investigator through the end of the study.

Descriptive statistics will be provided for clinical laboratory values (e.g., hematology, serum chemistry, coagulation studies, urinalysis) and VS data, presented as both actual values and changes from baseline relative to each on-study evaluation. Shift analyses will be conducted for selected laboratory parameters based on the baseline CTCAE grade to maximum CTCAE grade. Where applicable CTCAE terms do not exist, a grading system based on the upper and/or lower limits of normal will be used to classify the results.

No formal hypothesis-testing analysis of AE incidence rates will be performed.

Electrocardiogram analyses will include individual patient listings and summaries of abnormal and clinically significant ECG results. Actual values and changes from baseline in PR, QRS, and heart-rate corrected QT interval (QTc) intervals will be summarized by visit and dose arm. Full details of the QTc analysis including correction methods used will be described in the SAP.

Concomitant medications will be coded using the WHO Drug Dictionary. Concomitant medications prior to and after the start of the study drug will be listed by patient, and summarized by ATC term and dose arm.

### 12.5.4. Pharmacokinetic Analyses

Descriptive statistics will be used to summarize PK parameters for the parent compound AG-348 and the metabolite AGI-8702 for each dose group, and where appropriate, for the entire

population. Pharmacokinetic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum and maximum, geometric mean, and geometric coefficient of variation. PK analyses will be described in a separate PK SAP.

### 12.5.5. Pharmacodynamic Analyses

Descriptive statistics will be used to summarize PD parameters for 2,3-DPG and ATP for each dose group, and where appropriate for the entire population. Pharmacodynamic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation %. PD analyses will be described in a separate PD SAP.

### 12.5.6. Aromatase Hormone Analysis

The analyses of serum sex hormones will use appropriate graphic displays and statistical analyses to evaluate patient change from baseline for each parameter. These analyses will include summaries of actual values and change from baseline using appropriate descriptive statistics (mean, SD, median, min and max) as described above.

[redacted] These analyses will present information by each dose arm, and analyses of a pooled AG-348 cohort. Additional details regarding these analyses will be provided in the SAP.

### 12.5.7. Clinical Activity

Details on analyses to evaluate indicators of potential clinical activity of AG-348 in patients with PK deficiency will be described in the SAP. These will include changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation (serum iron/iron binding capacity). Characterization of Hb response which will include, but is not limited to percent of patients with increase in Hb, time to Hb response, and duration of Hb response will be explored.

[redacted]

## 12.6. Procedures for Reporting Deviations to Original Statistical Analysis Plan

All deviations from the original SAP will be provided in the final clinical study report.

# 13. ADMINSTRATIVE REQUIREMENTS

## 13.1. Good Clinical Practice

The study will be conducted in accordance with the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s). The Investigator will be thoroughly familiar with the appropriate use of the study drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study, and retained according to the appropriate regulations.

## 13.2. Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki (Appendix 15.4).

The Investigator must obtain IRB approval for the investigation and must submit written documentation of the approval to the Sponsor before he or she can enroll any patient into the study. The IRB will review all appropriate study documentation in order to safeguard the rights, safety, and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, Investigator's Brochure, informed consent, advertisements (if applicable), written information given to the patients, signed Dose Escalation Interim Safety Reports, annual progress reports, and any revisions to these documents will be provided to the IRB. The IRB is to be notified of any amendment to the protocol in accordance with local requirements. Progress reports and notifications of serious unexpected adverse drug reactions are to be provided to the IRB according to local regulations and guidelines.

## 13.3. Patient Information and Informed Consent

The Investigator or trained designee will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk, and benefit of the study. Patients must also be notified that they are free to discontinue from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

After the study has been fully explained, written informed consent will be obtained from the patient prior to study participation.

The patient's signed and dated informed consent must be obtained before conducting any study-related procedures. The Investigator must maintain the original, signed consent form. A copy of the signed form must be given to the patient.

The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

## 13.4. Patient Confidentiality

In order to maintain patient privacy, all source documents, study drug accountability records, study reports and communications will identify the patient by the assigned patient number. The Investigator will grant monitor(s) and auditor(s) from the Sponsor or its designee and regulatory authority(ies) access to the patient's original medical records for verification of data gathered on the source documents and to audit the data collection process. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

## 13.5. Protocol Compliance

The Investigator will conduct the study in compliance with the protocol. Modifications to the protocol should not be made without agreement of both the Investigator and the Sponsor. Changes to the protocol will require written IRB approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to patients. The IRB may provide, if applicable, where regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval/favorable opinion of the IRB. The Sponsor or designee will submit all protocol modifications to the regulatory authority(ies) in accordance with the governing regulations.

When immediate deviation from the protocol is required to eliminate an immediate hazard(s) to patients, the Investigator will contact the Sponsor's Medical Monitor (or Responsible Medical Officer), if circumstances permit, to discuss the planned course of action. Any departures from the protocol must be fully documented in the source documents/database.

## 13.6. Data Management

A paper or eCRF will be completed for each patient or an electronic data capture system will be used. The electronic data capture system (EDC) (Medidata Rave®) is a software tool designed to ensure quality assurance and facilitate data capture during clinical trials. Through a system regulated workflow that includes barcode scanning and interfaces to medical equipment to avoid manual data entry, study operations performance is controlled and captured in real time. The system is fully Code of Federal Regulations (CFR) 21 Part 11 compliant. Source documentation supporting the data should indicate participation in the study and should document the dates and details of study procedures, AEs, and patient status. The Investigator, or trained designee should complete and the Investigator should verify the source documents as the information is collected. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data. The Investigator will ensure the accuracy, completeness, and timeliness of the data reported to the Sponsor. Data collection processes and procedures will be reviewed and validated to ensure completeness, accuracy, reliability, and consistency. A complete audit trail will be maintained of all data changes. The Investigator or designee will cooperate with the Sponsor's representative(s) for the periodic review of study documents to ensure the accuracy and completeness of the data capture system at each scheduled monitoring visit. Electronic consistency checks and manual review will be used to identify any errors or inconsistencies in the data. This information will be provided to the respective study sites by means of electronic or manual queries. The Investigator or designee will prepare and maintain adequate and accurate study documents (medical records, ECGs, AE, and concomitant

medication reporting, raw data collection forms, etc.) designed to record all observations and other pertinent data for each patient receiving study treatment. The Investigator will allow Sponsor representatives, contract designees, authorized regulatory authority inspectors, and the IRB to have direct access to all documents pertaining to the study.

## 13.7. Source Document/Case Report Form Completion

Source documents will be completed for each study patient. It is the Investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the patient's source document. The source document should indicate the patient's participation in the study and should document the dates and details of study procedures, AEs and patient status.

The Investigator, or designated representative, should complete the source document as soon as possible after information is collected, preferably on the same day that a study patient is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.

The Investigator will sign and date each required assessment for all study patients. The Investigator will retain all completed source documents.

## 13.8. Direct Access to Source Data

The study will be monitored by the Sponsor or its designee. Monitoring will be done by personal visits from a representative of the Sponsor (site monitor) and will include on-site review of the source documents for completeness and clarity, cross-checking with source documents, and clarification of administrative matters will be performed. The review of medical records will be performed in a manner to ensure that patient confidentiality is maintained.

The site monitor will ensure that the investigation is conducted according to protocol design and regulatory requirements by frequent communications (letter, telephone, e-mail, and fax).

All unused study drug and other study materials are to be returned to the Sponsor or designee after the study has been completed and the database has been locked.

Regulatory authorities, the IRB, and/or the Sponsor's clinical quality assurance group or designee may request access to all source documents, database, and any other applicable study documentation for an on-site audit or inspection. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities.

## 13.9. Record Retention

The Investigator will maintain all study records according to ICH-GCP and applicable regulatory requirement(s). Records will be retained for at least 2 years after the last marketing application approval or 2 years after formal discontinuation of the clinical development of the investigational product or according to applicable regulatory requirement(s). If the Investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility. The Sponsor must be notified in writing if a custodial change occurs.

## 13.10. Liability and Insurance

The Sponsor has subscribed to an insurance policy covering, in its terms and provisions, its legal liability for injuries caused to participating persons and arising out of this research performed strictly in accordance with the scientific protocol as well as with applicable law and professional standards.

## 13.11. Publication of Study Findings and Use of Information

All information regarding AG-348 supplied by the Sponsor or designee to the Investigator is privileged and confidential information. The Investigator agrees to use this information to accomplish the study and will not use it for other purposes without consent from the Sponsor. It is understood that there is an obligation to provide the Sponsor with complete data obtained during the study. The information obtained from the clinical study will be used towards the development of AG-348 and may be disclosed to regulatory authority(ies), other Investigators, corporate partners, or consultants as required.

# 14. LIST OF REFERENCES

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Christensen RD, Yaish HM, Johnson CB, Bianchi P, and Zanella A. Six children with pyruvate kinase deficiency from one small town: molecular characterization of the PK-LR gene. *J Pediatr.* 2011;159(4):695-7.

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Ghidini A, Sirtori M, Romero R, Yarkoni S, Solomon L, and Hobbins JC. Hepatosplenomegaly as the only prenatal finding in a fetus with pyruvate kinase deficiency anemia. *Am J Perinatol.* 1991;8(1):44-6.

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Tanphaichitr VS, Suvatte V, Issaragrisil S, Mahasandana C, Veerakul G, Chongkolwatana V, et al. Successful bone marrow transplantation in a child with red blood cell pyruvate kinase deficiency. *Bone Marrow Transplant.* 2000;26(6):689-90.

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Zanella A, Fermo E, Bianchi P, and Valentini G. Red cell pyruvate kinase deficiency: molecular and clinical aspects. *Br J Haematol.* 2005;130(1):11-25.

# 15. APPENDICES

## 15.1. Schedules of Assessments

### Table 4: Schedule of Assessments:

| **Timing** | **Pre-Treatment** | **Month 1** | | | | **Months 2 and 3** | | | **Months 4, 5 and 6** | | | **Follow Up** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | **Baseline / D1** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day** | **-28 to -1** | **1** | **8**[1] | **15** | **22**[1] | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window** | | | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| Written Informed Consent | X | | | | | | | | | | | |
| PK enzyme assay (confirmation of PK deficiency) | X | | | | | | | | | | | |
| PKR Genotype (for randomization) | X | | | | | | | | | | | |
| UGT1A1 Genotype | X | | | | | | | | | | | |
| Demographics | X | | | | | | | | | | | |
| Medical/Surgical History (General and PK deficiency-specific)[2] | X | | | | | | | | | | | |
| Medication History | X | | | | | | | | | | | |
| Transfusion History | X | | | | | | | | | | | |
| Confirmation of Vaccinations (Splenectomized Patients) | X | | | | | | | | | | | |
| Physical Examination[3]/ Height[3] and Weight | X | X | | | X | | X | X | X | X | X | X |
| Performance Status | X | X | | | X | | X | X | X | X | X | X |
| Vital signs (BP, HR, RR, T)[4] | X | X | X | X | X | X | X | X | X | X | X | X |
| 12-lead ECG[5] | X | X | X | | X | | | | | | X | X |
| DXA Scan[6] | X | | | | | | | | | | X[7] | |
| Laboratory Evaluations[8]: | | | | | | | | | | | | |

| Timing | Pre-Treatment | Month 1 | | | | Months 2 and 3 | | | Months 4, 5 and 6 | | | Follow Up |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | **Baseline / D1** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day** | **-28 to -1** | **1** | **8**[1] | **15** | **22**[1] | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window** | | | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| HBsAg, HCV Ab, HIV1 and 2 Ab | X | | | | | | | | | | | |
| RBC antibody Screen | X | | | | | | | | | | | |
| Hematology (CBC)[9] | X | X | X | X | X | X | X | X | X | X | X | X |
| Haptoglobin[10] | | X | | | X | | | X | | | X | X |
| EPO levels[11] | | X | | | X | | | X | | | X | X |
| G6PD screen | X | | | | | | | | | | | |
| Serum Chemistry[12] | X | X | X | X | X | X | X | X | X | X | X | X |
| Iron Panel[13] | | X | | | | | | X | | | X | |
| Carboxyhemoglobin (COHb) | | X | | | X | X | X | X | X | X | X | |
| Coagulation Studies[14] | X | X | | | X | | | X | | | X | X |
| Urinalysis[15] | X | X | | | X | | | X | | | X | X |
| Serum or Urine Pregnancy[16] | X | X | | | | | | | | | | |
| Lipids[17] | | X | | | | X | | X | | | X | X |
| Hormonal Testing[18] | X | X | | | | | | X | | | X | X |
| Serum NTX and CTX | | X | | | | | | X | | | X | |
| Randomization[19] | X | | | | | | | | | | | |
| Study Drug Administration | | X | X | X | X | X | X | X | X | X | X | |
| Dispense Study Drug[20] | | X | X | X | X | X | X | X | X | X | | |
| PK blood sampling[21] | | X | | X | X | X | X | X | X | X | X | |
| PD Assessments[21] | | | | | | | | | | | | |
| 2,3 DPG/ATP | | X | | X | X | X | X | X | X | X | X | |

| **Timing** | **Pre-Treatment** | **Month 1** | | | | **Months 2 and 3** | | | **Months 4, 5 and 6** | | | **Follow Up** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | **Baseline / D1** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day** | **-28 to -1** | **1** | **8**[1] | **15** | **22**[1] | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window** | | | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| [redacted] | | | | | | | | | | | | |
| Adverse Events[23] | | Continuous | | | | | | | | | | X |
| Transfusion Record | X | X | X | X | X | X | X | X | X | X | X | X |
| Concomitant Medications | X | X | X | X | X | X | X | X | X | X | X | X |
| Rollover to extension study | | | | | | | | | | | X | |

Abbreviations: Ab = antibody; ATP = adenosine triphosphate; BP = blood pressure; CBC= complete blood count; COHb = carboxyhemoglobin; CTX = C-terminal telopeptide; D = day; DPG = diphosphoglycerate; DXA = Dual-energy x-ray absorptiometry; ECG = electrocardiogram; EPO = erythropoietin; FSH = follicle stimulating hormone; G6PD = glucose-6-phosphate-dehydrogenase; HBsAg = hepatitis B surface antigen; HCV Ab = hepatitis C virus antibody; HDL-C = high-density lipoprotein-C; HIV = human immunodeficiency virus; HR = heart rate; NTX = N-terminal telopeptide; PD = pharmacodynamic; PK = pharmacokinetic; PK deficiencyD = pyruvate kinase deficiency; PKR = pyruvate kinase isoform R; RR = resting rate; W = week.

*Whenever more than one assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (e.g., VS, ECG, blood draw). The timing of these assessments should allow the blood draw to occur at the exact nominal time. The order of procedures may be revised with prior discussion between Sponsor and site.*

1 In-clinic visits on Day 8 and Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis; in these instances PK/PD sampling would not be required and dispensing of study medication would not be performed.

2 Medical history, including all relevant prior medical history, current medical conditions, and hematology profile (CBCs) over prior 6 months, will be obtained at the Screening assessment.

3 A complete physical examinations (genital and rectal examinations will be performed at the discretion of the Investigator) will be obtained at Screening, Baseline, Week 12, and the Follow up Visit (Week 28), or Week 24 for patients rolling over into extension study. Limited focused physical examinations will be performed at all other specified visits. Height to be collected at Screening only.

4 Vital signs will include systolic and diastolic blood pressure, heart rate, respiratory rate, and temperature.

5 12-lead ECGs are to be conducted after 5 minutes of recumbency. Screening ECG will be performed at least 7 days prior to Day 1 dosing.

6 If a DXA scan of the hip and spine with T and Z scores has been performed within 3 months preceding the first day of dosing, this may be used to meet this requirement.

7 Week 24 DXA scan may be performed anytime between Weeks 24 and 28 and must be performed at the same imaging center on the same DXA machine as the original Screening DXA scan.

[8] Laboratory evaluations (hematology, serum chemistry, coagulation studies, and urinalysis) are to be collected in the morning. These should be collected following an overnight fast on Baseline Day 1 Week 6 (Day 43), Week 12 (Day 85), Week 24 (Day 169), and Follow-up Week 28 (Day 197), when the lipid samples are also included.

[9] Complete blood count (CBC) to be performed at Screening and prior to dosing on Day 1 and will include HCT, Hb, red blood cell (RBC) count, absolute reticulocyte count, percent reticulocyte count, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count with manual differential, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and platelet count

[10] Haptoglobin will be performed prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

[11] Erythropoietin (EPO) levels will be performed prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

[12] Alkaline phosphatase, sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide (CO2), albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and indirect bilirubin.

[13] Iron (Fe), total iron-binding capacity (TIBC), percent saturation, and ferritin will be performed prior to dosing on Day 1, at the end of Week 12 and at the end of Week 24.

[14] Fibrinogen, activated partial thromboplastin time (aPTT), and international normalized ratio (INR) will be performed at Screening, prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

[15] Color, appearance, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, and occult blood. Microscopic inspection of sediment should only be performed for cause or to investigate an abnormal dipstick finding per the Investigator's discretion. Urinalysis will be performed at Screening, prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

[16] Must be repeated at any point throughout the study period if pregnancy is clinically suspected.

[17] Total cholesterol, HDL-C, and triglyceride samples will be collected in the morning following an overnight fast.

[18] Three Screening/Baseline samples will be collected for estradiol and testosterone (total and free). Samples will be collected in the AM on 3 different days; the samples collected on Baseline/Day 1 may comprise one of these. Serum estrone, estradiol, and free and total testosterone will then follow the schedule indicated on Weeks 12, 24, and 28. FSH will only be performed at Screening for female patients only for confirmation of post-menopausal status.

[19] Randomization will be performed following PKR genotyping and prior to dosing on Day 1.

[20] Study drug will be dispensed on a 28-day schedule, or on an alternate schedule (< 28 days) as needed to accommodate patient visit schedule and dose modifications.

[21] For the first 10 patients treated, extensive PK/PD sampling will be conducted on Days 1 and 15 (see Appendix 15.1, Table 5 for details), followed by limited PK/PD sampling from Week 3 to Week 24 (see Appendix 15.1, Table 6 for details). Limited PK/PD sampling will be conducted on the remainder of patients treated (see Appendix 15.1 , Table 6). See Section 10.6.1, Section 10.7, and Section 10.9 for details on blood sampling for PK and PD assessments, respectively, and guidelines on sample processing and storage.

[redacted]

[23] All patients will be evaluated for AEs from the time they sign informed consent until they complete all scheduled study follow-up visits or withdraw consent, whichever occurs first.

**Table 5: Schedule of Assessments: Extensive PK/PD Sampling**

| Sample Timing/Interval | Month 1 | | | | | | | | Months 2 and 3 | | | Months 4, 5 and 6 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Visit | Baseline / D1<br>W2 / D15 | | | | | | | W3 | W6 | W9 | W12 | W16 | W20 | W24 |
| Study Day | 1/15 | | | | | | | 22 | 43 | 64 | 85 | 113 | 141 | 169 |
| Visit Window | ± 2 D (D15) | | | | | | | ± 2 D | ± 7 D | ± 7 D | ± 7 D | ± 7 D | ± 7 D | ± 7 D |
| Timing | Pre-dose[1] | 30 min[2] | 1 hr[2] | 2 hr[2] | 4 hr[3] | 8 hr[3] | 12 hr[3] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] |
| PK blood sample | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| 2,3 DPG/ATP | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| [redacted] | | | | | | | | | | | | | | |

Abbreviations: ATP = adenosine triphosphate; D = day; DPG = diphosphoglycerate; PD = pharmacodynamic; PK = pharmacokinetic; PKR = pyruvate kinase isoform R; W = week.

[1] The acceptable time window will be within 60 minutes prior to study treatment dose administration for the pre-dose PK/PD sample.
[2] The acceptable time window will be within ± 5 minutes of the scheduled collection time for the 30 minute, 1 and 2 hour PK/PD samples.
[3] The acceptable time window will be within ± 30 minutes of the scheduled collection time for the 4, 8, and 12 hour PK/PD samples.
[redacted]
[5] If the 12 hour time point cannot be collected at site, an 8 hour time point may be collected instead.

**Table 6: Schedule of Assessments: Limited PK/PD Sampling**

| Sample Timing/Interval | Month 1 | | | Months 2 and 3 | | | Months 4, 5 and 6 | | |
|---|---|---|---|---|---|---|---|---|---|
| Visit | Baseline / D1 | W2 | W3 | W6 | W9 | W12 | W16 | W20 | W24 |
| Study Day | 1 | 15 | 22 | 43 | 64 | 85 | 113 | 141 | 169 |
| Visit Window | - | ± 2 D | ± 2 D | ± 2 D | ± 7 D | ± 7 D | ± 7 D | ± 7 D | ± 7 D |
| Timing | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] |
| PK blood sample | X | X | X | X | X | X | X | X | X |
| 2,3 DPG/ATP | X | X | X | X | X | X | X | X | X |
| [redacted] | | | | | | | | | |

Abbreviations: ATP = adenosine triphosphate; D = day; DPG = diphosphoglycerate; PD = pharmacodynamic; PK = pharmacokinetic; PKR = pyruvate kinase isoform R; W = week.

[1] The predose blood sample for plasma PK/PD analysis should be collected within 60 minutes prior to study treatment dose administration.

## 15.2. National Cancer Institute Common Terminology Criteria for Adverse Events

The NCI CTCAE, Version 4.03, can be accessed using the following link:

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

## 15.3. Potential Drug Interactions

Strong inhibitors of CYP3A4 (listed in Table 7) are not permitted for use with AG-348. Based on modeling of AG-348 metabolism and tolerability data from the Phase 1 MAD study, it is thought that moderate inhibitors of CYP3A4 do not pose a risk to patients dosed with AG-348.

In-vivo data from the Phase 1 MAD show AG-348 induces CYP3A4 and induces its own metabolism, presumably via CYP3A4. The use of strong CYP3A4 inducers in combination with AG-348 is expected to reduce AG-348's efficacy. Therefore, administration of strong CYP3A4 inducers (listed in Table 8) with AG-348 is not permitted. Strong inhibitors of drug transport (listed in Table 9) are not permitted for use with AG-348.

Digoxin is not permitted for use with AG-348.

Induction of CYP3A4 by AG-348 is expected to reduce the efficacy of certain sensitive co-medications. Co-administration of AG-348 with the drugs in Table 10 should be done with caution, as their efficacy may be reduced.

Of note, in accordance with Inclusion Criteria 14, women in the trial utilizing oral contraception must utilize barrier methods while taking AG-348.

The expected patient co-medications deferoxamine, deferasirox and oral penicillin are not expected to interact with AG-348.

**Table 7: Strong and Moderate CYP3A4 Inhibitors**

| Strong CYP3A4 Inhibitors: Contraindicated | Moderate CYP3A4 Inhibitors: No Action |
|---|---|
| Indinavir | Aprepitant |
| Nelfinavir | Erythromycin |
| Ritonavir | Fluconazole |
| Clarithromycin | Grapefruit juice[1] |
| Itraconazole | Verapamil |
| Ketoconazole | Diltiazem |
| Nefazodone | |
| Saquinavir | |
| Suboxone | |
| Telithromycin | |

Strong Inhibitor; > 5 fold increase in AUC
Moderate Inhibitor; > 2 fold, < 5 fold increase in AUC
[1] Although classified as a moderate CYP3A4 inhibitor, grapefruit and grapefruit juice are prohibited.

**Table 8: Strong CYP3A4 Inducers**

| **Strong CYP3A4 Inducers: Contraindicated** | |
|---|---|
| Efavirenz | Phenytoin |
| Nevirapine | Pioglitazone |
| Carbamazepine | Rifabutin |
| Glucocorticoids | Rifampin |
| Modafinil | St. John's Wort |
| Oxcarbazepine | Troglitazone |
| Phenobarbital | |

**Table 9: Strong P-glycoprotein Inhibitors**

| **Strong P-gp Inhibitors: Contraindicated** | |
|---|---|
| Amiodarone | Felodipine |
| Azithromycin | Itraconazole |
| Captopril | Ketoconazole |
| Carvedilol | Lopinavir |
| Clarithromycin | Ritonavir |
| Conivaptan | Quercetin |
| Cyclosporine | Quinidine |
| Diltiazem | Ranolazine |
| Dronedarone | Ticagrelor |
| Erythromycin | Verapamil |

**Table 10: Sensitive CYP3A4 Substrates**

| **Sensitive CYP3A4 Substrates: Substitute or Use with Caution** | | | | |
|---|---|---|---|---|
| **Macrolide antibiotics:** | **Prokinetic:** | **Steroid 6beta-OH:** | | |
| Clarithromycin | Cisapride | Estradiol | Finasteride | Terfenadine |
| Erythromycin | | hydrocortisone | Gleevec | Torisel |
| Telithromycin | **Antihistamines:** | progesterone | Haloperidol | Trazodone |
| | Astemizole | Testosterone | Irinotecan | Vemurafenib |
| **Anti-arrhythmics:** | Chlorpheniramine | | LAAM | Vincristine |
| Quinidine→3-OH | Terfenadine | **Miscellaneous:** | Lidocaine | Zaleplon |
| | | Alfentanil | Methadone | Ziprasidone |
| **Benzodiazepines:** | **Calcium Channel Blockers:** | Aprepitant | Nateglinide | Zolpidem |
| Alprazolam | Amlodipine | Aripiprazole | Nevirapine | |
| Diazepam→3OH | Diltiazem | Boceprevir | Ondansetron | |
| Midazolam | Felodipine | Buspirone | Pimozide | |
| Triazolam | Lercanidipine | Carbamazepine | Propranolol | |
| | Nifedipine | Cafergot | Quetiapine | |
| **Immune Modulators:** | Nisoldipine | Caffeine→TMU | Quinine | |
| Cyclosporine | Nitrendipine | Cilostazol | Risperidone | |
| Tacrolimus (FK506) | Verapamil | Cocaine | Romidepsin | |
| | | Codeine-N-demethylation | Salmeterol | |
| **HIV Antivirals:** | **HMG CoA Reductase Inhibitors**: | Dapsone | Sildenafil | |
| Indinavir | Atorvastatin | Dexamethasone | Sirolimus | |
| Nelfinavir | Cerivastatin | Dextromethorphan | Sorafenib | |
| Ritonavir | Lovastatin | Docetaxel | Sunitinib | |
| Saquinavir | Simvastatin | Domperidone | Tamoxifen | |
| | | Eplerenone | Taxol | |
| | | Fentanyl | Telaprevir | |

## 15.4. Declaration of Helsinki

**WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI:**

Recommendations Guiding Medical Doctors in Biomedical Research Involving Human Subjects

Adopted by the 18th World Medical Association (WMA) General Assembly, Helsinki, Finland, June 1964 and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975, 35th WMA General Assembly, Venice, Italy, October 1983, and the 41st WMA General Assembly, Hong Kong, September 1989, the 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996; 52nd WMA General Assembly, Edinburgh, Scotland, October 2000; 53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added); 55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added); and 59th WMA General Assembly, Seoul, October 2008.

### A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.
   The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of subjects, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my subject will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the subject's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
8. In medical practice and in medical research, most interventions involve risks and burdens.

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.
10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

## B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.
12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, Sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.
15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the Sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any SAEs. No change to the protocol may be made without consideration and approval by the committee.
16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on subjects or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects

must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.
25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.
27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.
28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious subjects, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.
30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

## C ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the subjects who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:
    - The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
    - Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the subjects who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.
33. At the conclusion of the study, subjects entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.
34. The physician must fully inform the subject which aspects of the care are related to the research. The refusal of a subject to participate in a study or the subject's decision to withdraw from the study must never interfere with the subject-physician relationship.
35. In the treatment of a subject, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the subject or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

agios

# Clinical Study Protocol AG348-C-003

## A Phase 2, Open Label, Randomized, Dose Ranging, Safety, Efficacy, Pharmacokinetic and Pharmacodynamic Study of AG-348 in Adult Patients with Pyruvate Kinase Deficiency

| | |
|---|---|
| Study Sponsor: | Agios Pharmaceuticals, Inc.<br>88 Sidney Street, Suite 250<br>Cambridge, MA 02139-4169<br>Phone: 617-649-8600<br>Fax: 617-649-8618 |
| Responsible Medical Officer: | [redacted], MD, MPH & TM<br>Agios Pharmaceuticals, Inc.<br>Mobile Phone: [redacted]<br>Office Phone: [redacted]<br>Email: [redacted] |
| Study Medical Monitor | [redacted], MD<br>On behalf of Agios Pharmaceuticals, Inc.<br>Mobile Phone: [redacted]<br>Office Phone: [redacted]<br>Email: [redacted] |
| Document Version (Date): | Version 1.0 (05 January 2015) |

This study will be conducted according to the protocol and in compliance with Good Clinical Practice, the ethical principles stated in the Declaration of Helsinki, and other applicable regulatory requirements.

**CONFIDENTIALITY NOTE:**

The information contained in this document is confidential and proprietary to Agios Pharmaceuticals, Inc. Any distribution, copying, or disclosure is strictly prohibited unless such disclosure is required by federal regulations or state law. Persons to whom the information is disclosed must know that it is confidential and that it may not be further disclosed by them.

# INVESTIGATOR'S AGREEMENT

I understand that all documentation provided to me by Agios or its designated representative(s) concerning this study that has not been published previously will be kept in strict confidence. This documentation includes the study protocol, Investigator's Brochure, case report forms, and other scientific data.

This study will not commence without the prior written approval of a properly constituted Institutional Review Board (IRB). No changes will be made to the study protocol without the prior written approval of Agios and the IRB, except where necessary to eliminate an immediate hazard to the patient.

I have read, understood, and agree to conduct this study as outlined in the protocol and in accordance with the guidelines and all applicable government regulations.

| Investigator Name (printed) | Investigator Signature | Date |
|---|---|---|

Investigational site or name of institution and location (printed)

# 2. SYNOPSIS

**Name of Sponsor/Company:**
Agios Pharmaceuticals, Inc.

**Name of Investigational Product:**
AG-348

**Name of Active Ingredient:**
AG-348 sulfate hydrate

**Title of Study:**
A Phase 2, Open Label, Randomized, Dose Ranging, Safety, Efficacy, Pharmacokinetic and Pharmacodynamic Study of AG-348 in Adult Patients with Pyruvate Kinase Deficiency

**Study Center(s):**
This study will be conducted at multiple study centers.

**Phase of Development:** 2

***Objectives:***

**Primary:**

- Evaluate the safety and tolerability of up to 24 weeks of AG-348 administration in patients with pyruvate kinase deficiency (PK Deficiency).

**Secondary:**

- Evaluate the pharmacokinetics (PK) of AG-348 and the metabolite AGI-8702.
- Evaluate the pharmacodynamic (PD) response of adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG) after administration of AG-348.
- Evaluate indicators of clinical activity of AG-348 in patients with PK Deficiency, including changes in hemoglobin (Hb), hematocrit (HCT), reticulocyte count, haptoglobin (Hp), carboxyhemoglobin (COHb), lactate dehydrogenase (LDH), total and indirect bilirubin, erythropoietin (EPO), ferritin, and transferrin saturation (serum iron/iron binding capacity).

[redacted]

**Methodology:**
Study AG348-C-003 is a Phase 2, open label, two arm, multicenter, randomized, dose-ranging study during which adult patients with PK Deficiency will receive multiple doses of AG-348 for up to 24 weeks. Patients with PK Deficiency confirmed by red blood cell PK enzymatic assay performed at Screening will be eligible to participate in this study. At Week 25, patients who safely tolerate AG-348 and demonstrate clinical activity of AG-348 may be eligible to immediately roll over to a separate safety extension study for continued treatment. Patients who finish treatment at the end of 24 weeks or sooner will undergo follow-up assessment 4 weeks after the last dose of study drug. Patients with toxicity suspected to be related to study drug will continue follow-up until the adverse event (AE) resolves, is declared chronic by the Investigator, or the patient is lost to follow-up.

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| Initially, up to 25 patients will be randomized on an open-label 1:1 basis to each of two twice-daily (BID) doses of AG-348 (up to 50 patients total; see Figure 1, Study Schema). The dose of Arm 1 is 300 mg of AG-348 administered orally every 12 hours (q12h, BID). The dose of Arm 2 is 50 mg of AG-348 administered orally q12h (BID). Randomization will be stratified by *PKR* mutation in order to maintain balance as much as possible across the dose arms for the specific mutations expected to be most frequently enrolled. The *PKR* mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H) and all other mutations ("other"). Mutation status is defined by the presence of at least one of the indicated mutations; patients with more than one stratified mutation will be assigned based on Sponsor's discretion.<br><br>The doses for each arm have been selected from the forerunner AG348-C-001 single ascending dose (SAD) and AG348-C-002 multiple ascending dose (MAD) studies in healthy adult volunteers to represent the range of doses/exposures that were safely tolerated and resulted in maximal or near-maximal PD effects on 2,3-DPG and ATP.<br><br>Because PK Deficiency is a rare disease with a limited eligible patient population and because the underlying pathophysiology and clinical phenotype of affected patients is heterogenous due to the wide variety of mutations in PKR that cause the disease, it is important to focus closely on dose findings in this first-in-patient study. Therefore, in addition to initiating this study with 2 different doses of AG-348 administered q12h, a Data Review Team (DRT) will be established to review study data on a regular basis and adapt the study design, dose and schedule of AG-348.<br><br>The DRT will monitor safety on an on-going basis and meet at regular intervals (approximately every 6 weeks), or *ad* hoc, as necessary, to review AEs, vital signs (VS), clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and electrocardiograms (ECGs) on enrolled patients. The DRT will also review available PK/PD data and indicators of clinical activity (e.g., changes from baseline in Hb). The DRT will be comprised of the study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer.<br><br>Approximately every 6 weeks beginning six weeks after the first patient is dosed or *ad hoc* as necessary, the DRT will review cumulative safety data, available PK/PD data, and clinical activity data. Based on the DRT's recurring 6 week reviews, the DRT may exercise one or more of the following options:<br>• Continue treatment and enrollment in existing arms without change.<br>• Add 1 new dose arm (Arm 3) to enroll up to 25 patients at a dose to be determined; the dose for Arm 3 may be lower or higher than Arm 1 and Arm 2 doses, but will not exceed 360 mg q12h; and the dose regimen may be less frequent than q12h.<br>• Terminate or suspend enrollment to allow further review of clinical data in Arm 1 and/or Arm 2 (and/or potential Arm 3). Enrollment in an arm could be terminated or suspended to allow further review, for example, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity.<br>• Re-assign patients' doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the patients in the terminated arm will remain in their original arm, i.e., they will not count towards the enrollment quota of the arm whose dose and |

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schedule is being adopted.

- Implement specific genotype restrictions to enrollment in one or more arms to ensure representation of patients with genotypes of greatest clinical relevance.

The DRT will perform these evaluations on a recurring 6-week basis. The data that the DRT will review to make these decisions is expected to include, but are not necessarily limited to, the following:

- *Safety Observations:* all AEs; VS, clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and ECG;
- *PK and PD Observations:* including changes in 2,3-DPG and ATP;
- *Indicators of Clinical Activity:* including changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, EPO, total and indirect bilirubin, ferritin, and transferrin saturation.

If a third dose arm is implemented, the dose of AG-348 selected will not exceed 360 mg BID, as this was the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy volunteers. The PK/PD sampling schedule in a potential third dosing arm will be determined by Sponsor's discretion and may follow either the extensive or limited PK/PD sampling schedules as specified.

The DRT will monitor the data in an ongoing manner as described and may make a decision to terminate enrollment in an arm if unacceptable AEs are observed, PD activity is limited, and/or markers of clinical activity indicate no effect. Unacceptable AEs are defined as ≥ Grade 3 AEs (using National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAEv4.03]) that are assessed as at least possibly related to AG-348, with the exception of Grade 3 headache, hot flash/flushing, nausea, vomiting, and/or diarrhea that is transient in duration (< 24 hours) or able to be medically managed to ≤ Grade 2 within 24 hours. The DRT may also exercise discretion to terminate enrollment in an arm if it observes a frequency of Grade 2 AEs that would make it difficult to support long-term dosing.

As the number of enrolling arms changes in the study (for example, from 2 to 3), the randomization scheme will adjust to enable balanced randomization into each actively accruing arm. Randomization and stratification will cease in the event that only a single arm is left enrolling.

Depending on possible early termination of 1 or both of the initial 2 arms, or the addition of a third arm, the study could enroll up to a maximum of 75 patients.

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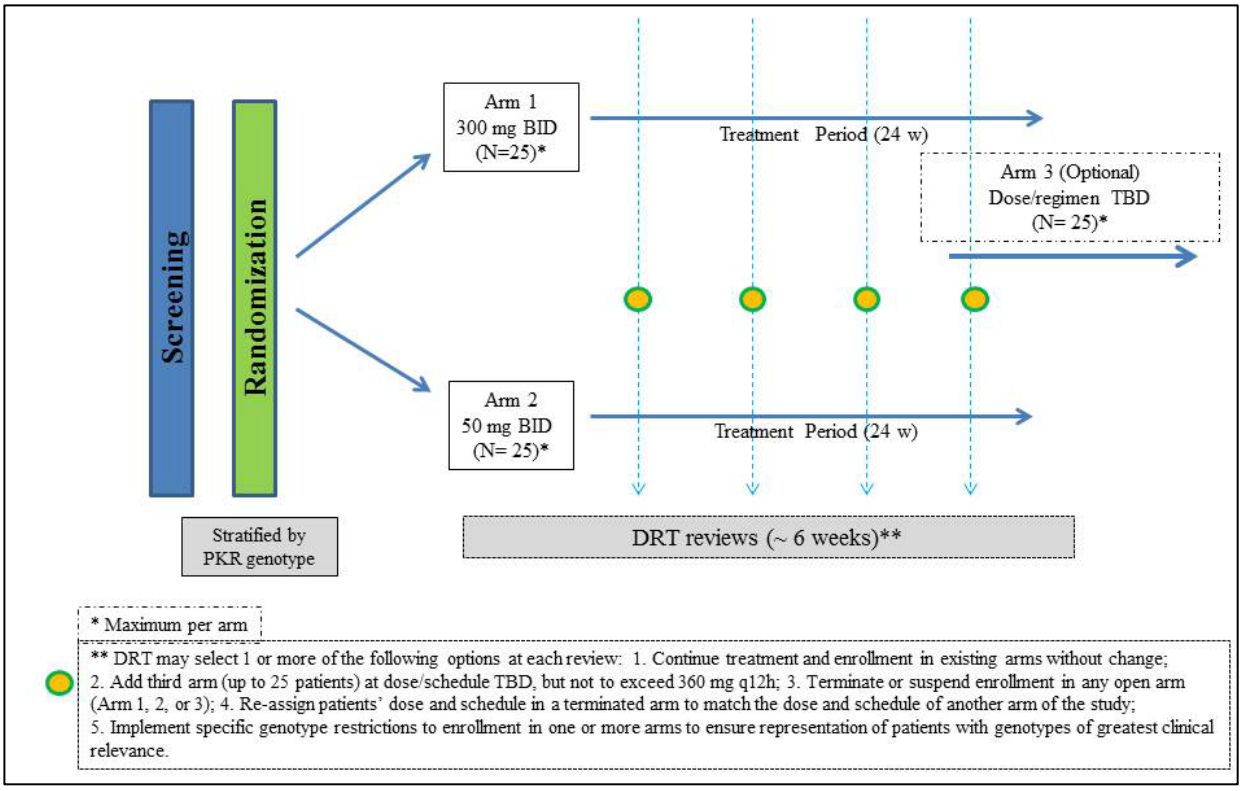
**Figure 1: Study Schema**




Abbreviations: BID (q12h) = twice-daily (every 12 hours); DRT = data review team; PKR = pyruvate kinase red blood cell isoform; TBD= to be determined; w = week(s).

***Visit Schedule***

Screening assessments will occur within 28 days prior to the first dose of study treatment. During the Treatment period, patients will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12), and monthly through Week 24 (Weeks 16, 20, and 24). Patients who safely tolerate AG-348 and demonstrate evidence of clinical activity of AG-348 through Week 24 may be eligible to immediately enter a separate extension study for continued treatment. For patients who finish treatment at the end of 24 weeks or sooner, or who elect not to enter the extension trial, study discharge will occur 4 weeks (Week 28 or earlier) following the last dose of study treatment at the final follow-up assessment.

**Dose Modifications for Safety and/or Increase in Hb Level**

The Investigator will monitor all patients for safety and tolerability. Modification of an individual patient's dose of AG-348 will be based on AEs and/or observed changes in Hb level as described in Section 9.7.1 and Section 9.7.2.

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**Number of patients (planned):** Up to approximately 75 patients.

**Diagnosis and main criteria for inclusion:**

**Inclusion criteria:**

For entry into the study, patients must meet all of the following criteria during the Screening or other specified period:

1. Signed written informed consent obtained prior to performing any study procedure, including screening procedures.
2. Male or female, aged 18 years and older.
3. Known medical history of PK Deficiency.
4. All patients must have documented clinical laboratory confirmation of PKD by red blood cell pyruvate kinase enzymatic assay performed at Screening by a designated central laboratory. Patients with prior documentation of PK Deficiency by red blood cell (RBC) enzymatic assay will have a reconfirmation of this result during Screening as a condition of enrollment.
    a. In the event that a patient's screening pyruvate kinase enzymatic assay is negative (i.e., shows normal pyruvate kinase activity), the patient will be eligible for enrollment if the genotyping shows a mutant genotype that has been previously documented in the literature to be associated with pyruvate kinase deficiency. If the genotyping shows a previously undescribed mutation in the PKR gene, then the eligibility for enrollment will be determined on a case-by-case basis by the Coordinating Investigator and Medical Monitor in discussion with the Investigator. If no mutation is defined, then the patient will not be eligible.
5. All patients must have genotypic characterization of the mutant PKR gene performed by a designated central laboratory at Screening, unless genotype is available from the patient's participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480).
6. All patients must have genotypic characterization of the UGT1A1 gene performed by a designated central laboratory to document whether they may have underlying Gilbert's Disease. Patients with Gilbert's Disease are eligible to enroll.
7. Males must have Hb $\leq$ 12.0 g/dL; females must have Hb $\leq$ 11.0 g/dL.
8. All patients must be considered transfusion independent as defined by: no greater than 3 units of RBCs transfused in the 12-month period up to the first day of study dosing and no transfusions within 4 months of first day of study dosing. Patients who have received more transfusion support than described above will evaluated for eligibility on a case-by-case basis by the Medical Monitor.

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9. Splenectomized patients:
    a. Must have undergone their procedure at least 6 months prior to Screening.
    b. Must be current in their vaccinations for Pneumococcal Conjugate (PCV13), Pneumococcal Polysaccharide (PPSV23), Quadrivalent Meningococcal vaccine, and *Haemophilus influenzae* type b (Hib) as recommended by Centers for Disease Control and Prevention's (CDC) Advisory Committee on Immunization Practices (ACIP) or immunization advisory groups in Canada and the European Union (for patients enrolled in Canada and the EU). [http://www.cdc.gov/vaccines/schedules/downloads/adult/adult-schedule.pdf] [Any missing vaccinations may be administered during the screening period.]
10. Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 2.
11. Patients must be taking at least 1 mg of folic acid daily for at least 21 days prior to first dose and continue daily during study participation.
12. Adequate organ function, defined as:
    a. Serum aspartate transaminase (AST) and alanine aminotransferase (ALT) ≤ 1.5 × upper limit of normal (ULN) (unless the increased AST is assessed by the Investigator as due to hemolysis).
    b. Normal or elevated levels of serum bilirubin. In patients with serum bilirubin > ULN, the elevation must be attributed to hemolysis with or without Gilbert's syndrome and must not be choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease.
    c. Serum creatinine ≤ 1.25 × ULN. If serum creatinine > 1.25 × ULN, then 24-hour measured or calculated (Cockcroft-Gault) glomerular filtration rate (GFR) ≥ 60 mL/min.
    d. Absolute neutrophil count (ANC) > $1.0 \times 10^9$/L.
    e. Platelet count ≥ $100 \times 10^9$/L.
    f. Activated partial thromboplastin time (aPTT) and international normalized ratio (INR) ≤ 1.25 × ULN, unless the patient is receiving therapeutic anticoagulants.
13. Women of childbearing potential (WOCBP) must agree to abstain from sexual intercourse or to use an acceptable/effective method of contraception (i.e., condom plus spermicide, condom plus oral contraceptive, condom plus intrauterine device [IUD], condom plus diaphragm with spermicide) from as soon as feasible during the Screening period until 30 days following the last dose of AG-348.
    a. WOCBP include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, and/or bilateral oophorectomy) or is not post-menopausal. Post-menopausal is defined as:
        i. Amenorrhea ≥ 12 consecutive months without another cause, and a documented serum follicle stimulating hormone (FSH) level > 35 mIU/mL;
        ii. Amenorrhea ≥ 12 consecutive months in women ≥ 62 years old (FSH testing is

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not required).

14. WOCBP must have a negative serum or urine pregnancy test within 72 hours before start of AG-348 dosing.
15. Women must not be breastfeeding.
16. Male patients, with the exception of those who have undergone vasectomy at least 6 months prior to Screening, must agree to abstain from sexual intercourse or, if sexually active, to use a condom with spermicide as contraception (regardless of their female partner's childbearing potential or their partner's use of their own contraception) from Day 1 of dosing until 30 days following the last dose of AG-348.

**Exclusion criteria:**

Patients who meet any of the following criteria at Screening or prior to dosing on Day 1 will not be enrolled in the study:

1. Hemoglobin level > 12.0 g/dL if male; Hb > 11.0 g/dL if female.
2. Additional diagnosis of any other congenital or acquired blood disorder, including glucose-6-phosphate-dehydrogenase (G6PD) deficiency, or any other hemolytic anemia process except for mild allo-immunization as a consequence of transfusion therapy.
3. Iron overload (hemosiderosis or concurrent hemochromatosis) sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac, hepatic, or pancreatic insufficiency.
4. Prior bone marrow or stem cell transplant.
5. Clinically symptomatic cholelithiasis or cholecystitis. (Prior cholecystectomy is not exclusionary. Patients with symptomatic cholelithiasis or cholecystitis may be rescreened once the disorder has been treated and clinical symptoms have resolved.)
6. Currently enrolled in another therapeutic clinical trial involving on-going therapy with any investigational or marketed product or placebo. Concurrent participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480) is permitted.
7. Exposure to any investigational drug, device, or procedure within 28 days prior to Screening.
8. Concurrent medical condition that could compromise participation in the study such as:
    a. Poorly controlled hypertension (defined as systolic blood pressure (BP) > 150 mm Hg or diastolic BP > 90 mm Hg) refractory to medical management.
    b. History of recent (within < 6 months from Screening date) congestive heart failure; myocardial infarction or unstable angina pectoris; or hemorrhagic, embolic, or

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thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.

c. Currently active infection requiring the use of parenteral anti-microbial agents or that is greater than Grade 3 (CTCAEv4.03) within 6 months of first dose.

d. A pattern or frequency of post-splenectomy sepsis that in the assessment of the Investigator could reasonably be expected to interfere with the ability of the patient to complete the 24 week study participation.

e. Positive test for hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV) antibody with signs of active Hepatitis B or C virus infection.

f. Positive test for human immunodeficiency virus (HIV) 1 or 2 antibody.

g. Diabetes mellitus judged to be in poor control by the Investigator or requiring > 3 anti-diabetic agents counting insulin (all insulins are considered one agent); use of insulin per se is not exclusionary.

h. History of any primary malignancy with the exception of: curatively treated non-melanomatous skin cancer; curatively treated cervical or breast carcinoma in situ; or other primary tumor treated with curative intent and no known active disease present and no treatment administered during the last 3 years.

9. Undergone major surgery within 6 months of first dose.

10. Current or recent history of psychiatric disorder that in the opinion of the Investigator or Medical Monitor could compromise the ability of the patient to cooperate with study visits and procedures.

11. Use of any of the restricted list of products known to strongly inhibit CYP3A4 drug metabolism (Appendix 15.3, Table 7) within 5 days prior to Day 1 dosing; or to strongly induce CYP3A4 metabolism (Appendix 15.3, Table 8) within 28 days prior to Day 1 dosing; or to strongly inhibit P-glycoprotein (P-gp) transporter (Appendix 15.3, Table 9) within 5 days prior to Day 1 dosing; or digoxin within 5 days prior to Day 1 dosing.

12. Serum bilirubin > ULN attributable to factors other than hemolysis and/or Gilbert's syndrome.

13. Male patients with heart-rate corrected QT (Fridericia's correction factor) QTcF interval > 450 msec, or female patients with QTcF interval > 470 msec with the exception of patients with a left bundle branch block (LBBB). Medical Monitor approval needed in patients with a LBBB.

14. Cardiac dysrhythmias judged as clinically significant by the Investigator or requiring therapy with drugs that are primarily substrates of CYP3A4.

15. History of allergy to sulfonamides if characterized by acute hemolytic anemia, anaphylaxis, or

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rash of erythema multiforme type or Stevens-Johnson syndrome.

16. Any other medical or psychological condition deemed by the Investigator to be likely to interfere with a patient's ability to understand and sign informed consent; cooperate with study visits, tests, and procedures; or otherwise safely and reliably participate in the study.

**Investigational product, dosage, and mode of administration:**
AG-348 sulfate hydrate capsules will be provided as 25 mg or 100 mg (free-base equivalent) of AG-348 sulfate hydrate without excipients in Swedish orange (25 mg) or white opaque (100 mg) gelatin capsules (size 2 capsules for all dose strengths). AG-348 will be administered orally BID. The number of capsules per dose will vary by assigned dose group. Patients will receive multiple oral (PO) doses of AG-348 over a 24-week treatment period. AG-348 will be administered with water and may be administered with or without food.

**Reference therapy, dosage and mode of administration:**
Not applicable.

**Duration of treatment:**
The duration of treatment for all patients on this study will be up to 24 weeks. Patients who safely tolerate and demonstrate one or more indicators of clinical activity of AG-348 may be eligible to immediately roll over to a separate safety extension study for continued treatment.

**Criteria for evaluation:**

**Safety:**
Monitoring of AEs, including determination of serious adverse events (SAEs) and AEs leading to discontinuation; safety laboratory parameters (hematology, chemistry, urinalysis, coagulation); physical examination findings; VS; and 12-lead ECGs. Adverse events will be graded using CTCAE, Version 4.03. Serum sex hormone levels (testosterone, estrone, and estradiol), bone turnover markers (serum N-terminal telopeptide [NTX] and serum C-terminal telopeptide [CTX]), total cholesterol, high-density lipoprotein-C (HDL-C), and triglycerides will be monitored for evidence of potential inhibition of aromatase by AG-348

**Indicators of Clinical Activity:**
Monitoring of potential indicators of clinical activity will include evaluating changes in Hb, HCT, reticulocyte count, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation.

**Pharmacokinetics:**
Approximately the first 10 patients treated, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 15.1, Table 5. The remainder of treated patients will undergo limited PK sampling as detailed in Appendix 15.1, Table 6. Serial blood sampling for determination of concentration-time profiles of AG-348 and its metabolite AGI-8702 will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of AG-348 and AGI-8702 will be obtained. AG-348 and AGI-8702 will be analyzed using qualified assays to determine concentrations in plasma. Pharmacokinetic parameters on Day 1 and Day 15 will be computed using standard non-

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compartmental methods based on observed plasma AG-348 and AGI-8702 concentrations.

**Pharmacodynamics:**

Pharmacodynamic assessments will include 2,3-DPG, ATP (secondary objectives), [redacted]. Approximately the first 10 patients treated will undergo extensive PD sampling as detailed in Appendix 15.1, Table 5. The remainder of treated patients will undergo limited PD sampling as detailed in Appendix 15.1, Table 6. Serial blood sampling for determination of levels of ATP and, 2,3-DPG will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of ATP and 2,3-DPG will be obtained. Adenosine triphosphate and 2,3 DPG will be analyzed using qualified assays to determine concentrations in whole blood. Pharmacodynamic parameters on Day 1 and Day 15 will be computed based on observed whole blood ATP and 2,3-DPG concentrations.

[redacted]

**Statistical methods:**

The primary objective of this Phase 2 study is to evaluate the safety and tolerability of up to 24 weeks of AG-348 in patients with PK Deficiency. Therefore, analyses will be primarily descriptive in nature; no formal hypothesis testing will be conducted. All analyses will be conducted separately within each dose arm, or pooled when appropriate.

Summaries will be produced for disposition, baseline disease characteristics and demographic data including genotype, safety measurements, PK, PD parameters and indicators of clinical activity. Data from each AG-348 dose group will be analyzed separately, and pooled across all dose groups where appropriate. All data will also be listed by individual patient. Categorical variables will be summarized by frequency distributions (number and percentages) and continuous variables will be summarized by descriptive statistics (mean, standard deviation [SD], median, minimum, and maximum).

Populations for analysis will include a Safety Analysis Set, a PK Analysis Set, and an Efficacy Analysis Set. The Safety Analysis set will include all patients who are enrolled and receive any dose of study treatment; this population will be the primary set for the analysis of safety data and the default analysis set for all data analyses unless otherwise stated. Patients will be classified according to treatment received, where treatment received is defined as the assigned treatment if it is received at least once, or as the first treatment received if assigned treatment is never received. The PK Analysis Set will include all patients in the Safety Analysis Set with sufficient plasma sample data to assess PK parameters. Results of the potential PD activity of AG-348 will also be based on the PK analysis set. The Efficacy Analysis Set will include all patients who are enrolled and achieve at least 50% compliance with their

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assigned dose intensity for at least 4 weeks of continued dosing. Additional efficacy analyses may be performed on subsets of patients with other degrees of compliance with their assigned dose intensity and/or duration of dosing. The Efficacy Analysis Set will be the primary set for the analysis of preliminary clinical activity data. Patients will be classified according to assigned treatment. [redacted]

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and the incidence of treatment-emergent AEs (TEAEs) (new or worsening from baseline) will be summarized by primary system organ class (SOC), preferred term (PT), CTCAE Version 4.03 severity, outcome, action taken with study drug, and relationship to study drug by dose group. Separate summaries will be produced for all TEAEs, treatment-related AEs (those considered by the Investigator as at least possibly drug related), SAEs, discontinuations due to AEs, and AEs ≥ Grade 3 severity. Individual patient listings will be provided for any deaths, SAEs, AEs leading to interruption and/or reduction of study drug dose, and AEs leading to discontinuation of treatment. Analyses of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined as any AE with onset beginning at the day of first administration of study drug, throughout the treatment period until 30 days after the last dose of study drug, or any event that was present at baseline but worsened in intensity or was subsequently considered drug-related by the Investigator through the end of the study.

Descriptive statistics will be provided for clinical laboratory values (e.g., hematology, serum chemistry, coagulation studies, urinalysis) and VS data, presented as both actual values and changes from baseline relative to each on-study evaluation. Shift analyses will be conducted for selected laboratory parameters based on the baseline CTCAE grade to maximum CTCAE grade. Where applicable CTCAE terms do not exist, a grading system based on the upper and/or lower limits of normal will be used to classify the results.

Electrocardiogram analyses will include individual patient listings and summaries of abnormal and clinically significant ECG results. Actual values and changes from baseline in PR, QRS, QTc intervals will be summarized by visit and dose arm.

Concomitant medications will be coded using the WHO Drug Dictionary. Concomitant medications prior to and after the start of the study drug will be listed by patient, and summarized by ATC term and dose arm.

Descriptive statistics will be used to summarize PK parameters for the parent compound AG-348 and the metabolite AGI-8702 for each dose group, and where appropriate, for the entire population. Pharmacokinetic parameters will be summarized using the following descriptive statistics: n, mean, standard deviation (SD), coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation.

Descriptive statistics will be used to summarize Pharmacodynamic parameters for 2,3-DPG and ATP for each dose group, and where appropriate for the entire population. Pharmacodynamic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation %.

Analyses of serum sex hormones will use appropriate graphic displays and statistical analyses to

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evaluate patient change from baseline for each parameter. These analyses will include summaries of actual values and change from baseline using appropriate descriptive statistics (mean, SD, median, min and max) as described above.

Analyses evaluating indicators of potential clinical activity of AG-348 in patients with PK Deficiency will include changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation (serum iron/iron binding capacity). Characterization of Hb response (e.g., % of patients whose Hb increases by a certain amount), as well as time to Hb response, and duration of Hb response will be explored, among others.

**Interim Review**

No formal statistical analysis will be conducted. Safety data will be reviewed on an ongoing basis by the DRT, who will meet to review safety, PK, PD, and preliminary clinical activity data at regular intervals (approximately every 6 weeks) throughout the duration of the study. The composition of the DRT, its meeting schedule, the data to be reviewed, and the decisions it is empowered to make have been described previously.

Additional interim reviews of data may be conducted to support decision making concerning the current clinical study, the sponsor's development programs in general, or in case of any safety concerns.

**Sample Size**

Due to the rare disease setting, the minimal sample size may be determined by feasibility. Depending on possible early termination of 1 or both of the initial 2 arms or the addition of a 3$^{rd}$ dose arm, the study could enroll up to a maximum of 75 patients.

# 3. TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

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# 4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

| Abbreviation or Specialist Term | Explanation |
|---|---|
| 2,3-DPG | 2,3-diphosphoglycerate |
| ACIP | Advisory Committee on Immunization Practices |
| ADME | Absorption, distribution, metabolism, excretion |
| ADP | Adenosine diphosphate |
| AE | Adverse event |
| ALC | Absolute lymphocyte count |
| ALP | Alkaline phosphatase |
| ALT | Alanine aminotransferase |
| ANC | Absolute neutrophil count |
| aPTT | Activated partial thromboplastin time |
| AST | Aspartate aminotransferase |
| ATP | Adenosine triphosphate |
| AUC | Area under the curve |
| $AUC_{0\text{-}12hr}$ | Area under the plasma concentration versus time curve from 0 to 12 hours |
| $AUC_{0\ 24hr}$ | Area under the plasma concentration versus time curve from 0 to 24 hours |
| $AUC_{0\text{-}\infty}$ | Area under the plasma concentration versus time curve from 0 to infinity |
| BCRP | Breast cancer resistance protein |
| BID | Twice-daily |
| BMI | Body mass index |
| BP | Blood pressure |
| BUN | Blood urea nitrogen |
| CDC | Centers for Disease Control and Prevention |
| CFR | Code of Federal Regulations |
| $CL_P$ | Total body plasma clearance |
| $C_{max}$ | Maximum plasma concentration |
| $CO_2$ | Carbon dioxide |
| COHb | Carboxyhemoglobin |
| CTCAE | Common Terminology Criteria for Adverse Events |

| Abbreviation or Specialist Term | Explanation |
|---|---|
| CTX | Serum C-terminal telopeptide |
| CV | Cardiovascular |
| DDI | Drug-drug interaction |
| CYP | Cytochrome P450 |
| DILI | Drug-induced liver injury |
| DLT | Dose-limiting toxicity |
| DRT | Data review team |
| ECG | Electrocardiogram |
| ECOG | Eastern Cooperative Oncology Group |
| eCRF | Electronic case report form |
| EDC | Electronic Data Capture |
| EPO | Erythropoietin |
| F | Oral bioavailability |
| FDA | Food and Drug Administration |
| FSH | Follicle stimulating hormone |
| G6PD | Glucose-6-phosphate-dehydrogenase |
| GCP | Good Clinical Practice |
| GFR | Glomerular filtration rate |
| GLP | Good Laboratory Practice |
| Hb | Hemoglobin |
| HBsAg | Hepatitis B surface antigen |
| HCT | Hematocrit |
| HCV | Hepatitis C virus |
| HDL-C | High-density lipoprotein-C |
| HDPE | High density polyethylene |
| hERG | Human ether à-go-go related gene |
| Hib | *Haemophilus influenzae* type b |
| HIV | Human immunodeficiency virus |
| Hp | Haptoglobin |
| $IC_{50}$ | Concentration of drug that achieved half-maximal inhibition |
| ICH | International Conference on Harmonization |
| INR | International normalized ratio |

| Abbreviation or Specialist Term | Explanation |
|---|---|
| IRB | Institutional Review Board |
| IUD | Intrauterine device |
| IV | Intravenous |
| LDH | Lactate dehydrogenase |
| MAD | Multiple ascending dose |
| MCH | Mean corpuscular hemoglobin |
| MCHC | Mean corpuscular hemoglobin concentration |
| MCV | Mean corpuscular volume |
| MedDRA | Medical Dictionary for Regulatory Activities |
| mPKR | PKR mutants |
| MTD | Maximum tolerated dose |
| NCI | National Cancer Institute |
| NOEL | No-observed-effect-level |
| NOAEL | No-observed-adverse-effect-level |
| NTX | Serum N-terminal telopeptide |
| P-gp | P-glycoprotein |
| PCV13 | Pneumococcal Conjugate |
| PD | Pharmacodynamic |
| PEP | Phosphoenolpyruvate |
| PK | Pharmacokinetic |
| PK Deficiency | Pyruvate kinase deficiency |
| PKR | Pyruvate kinase isoform R |
| PO | Oral |
| PPSV23 | Pneumococcal polysaccharide |
| PR | The portion of the ECG wave from the beginning of the P wave to the beginning of the QRS complex |
| PT | Preferred term |
| q12h | Every 12 hours |
| q24h | Every 24 hours |
| QD | Once-daily |
| QRS | QRS interval on an electrocardiogram |
| QTc | Heart-rate corrected QT interval |
| QTcB | Corrected QT interval - Bazett correction formula |

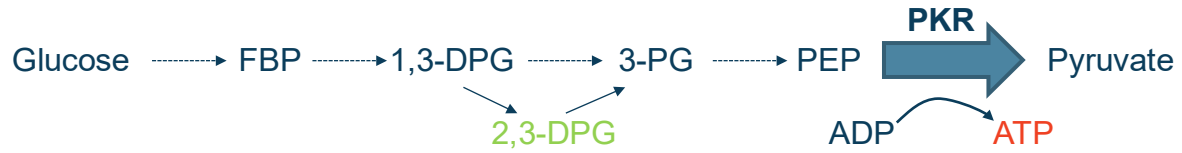
| Abbreviation or Specialist Term | Explanation |
|---|---|
| QTcF | Corrected QT interval - Fridericia's method |
| RBC | Red blood cell (count) |
| RDW | Red cell distribution width |
| SAD | Single ascending dose |
| SAE | Serious adverse event |
| SAP | Statistical analysis plan |
| SD | Standard deviation |
| SOC | System organ class |
| SOP(s) | Standard Operating Procedure(s) |
| $t_{½}$ | Apparent terminal half-life |
| TIBC | Total iron-binding capacity |
| $T_{max}$ | Time to maximum concentration |
| TEAE | Treatment-emergent adverse event |
| ULN | Upper limit of normal |
| VS | Vital signs |
| $V_{ss}$ | Volume of distribution at steady-state |
| Vz/F | Mean apparent volume of distribution |
| WBC | White blood cell |
| WMA | World Medical Association |
| WOCBP | Women of childbearing potential |
| WT | Wild type |

# 5. INTRODUCTION

## 5.1. Pyruvate Kinase Deficiency

Pyruvate kinase deficiency (PK Deficiency) is a glycolytic enzymopathy that results in non-spherocytic hemolytic anemia with a variable clinical presentation, ranging from mild or fully compensated forms to life-threatening neonatal anemia and life-long chronic hemolytic anemia associated with severe, debilitating co-morbidities. PK Deficiency is caused by mutations in the PKLR gene, which results in defective pyruvate kinase isoform R (PKR) in the red blood cell (RBC). As shown in the diagram below, PKR catalyzes the final and irreversible step in glycolysis, converting phosphoenolpyruvate (PEP) to pyruvate, and formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP). Mature RBCs rely almost exclusively on the process of glycolysis to generate the energy carrier molecule ATP. PKR is thus a key enzyme for maintaining energy homeostasis in erythrocytes, and it has been proposed that ATP levels are critical for optimally maintaining RBC membrane integrity (van Wijk and van Solinge 2005).

**Figure 2: Red Cell Glycolysis**




Abbreviations: 1,3 DPG = 1,3 diphosphoglycerate; 2,3 DPG = 2,3 diphosphoglycerate; 3-PG = 3-phosphoglycerate; ADP = adenosine diphosphate; ATP = adenosine triphosphate; FBP = fructose 1,6-bisphosphate; PEP = phosphoenolpyruvate; PKR = red blood cell-specific form of pyruvate kinase
Note: Not all steps in glycolysis are shown.

The majority of mutations in PKR that have been described have a deleterious effect on PKR catalytic activity, protein stability, and/or protein expression. PK Deficiency is associated with reduced RBC survival as well as impaired red cell maturation. Mature erythrocytes lack mitochondria relying predominantly on glycolysis to generate ATP. It is hypothesized that insufficient energy production promotes erythrocyte hemolysis due to impaired maintenance of cellular membrane homeostasis.

PK Deficiency is an autosomal recessive disease, in which both homozygotes and compound heterozygotes develop hemolytic anemia. More than 200 different mutations in the PKLR gene have been identified in patients with PK Deficiency to date, and the majority of these mutations are single nucleotide missense mutations (Zanella, et al. 2005). The prevalence of PK Deficiency, based on the most common pyruvate kinase mutation in Southern Europe, is estimated at 51 cases per million in the Caucasian population (Beutler and Gelbart 2000).

Some patients with PK Deficiency have a clinically severe disease course that can present as a severe hemolytic anemia in early infancy, requiring immediate care which typically includes blood transfusion support (Christensen, et al. 2011; Ghidini, et al. 1991; Hennekam, et al. 1990). Infants with PK Deficiency may have erythroblastosis fetalis with hepatosplenomegaly, extramedullary hematopoiesis, and severe indirect hyperbilirubinemia, which is associated with a high risk of kernicterus. Hyperbilirubinemia associated with the hemolytic state often requires

supportive treatment with intensive phototherapy. For other infants, the hyperbilirubinemia caused by hemolysis can require exchange transfusion, as well as supportive transfusion to keep their hematocrit (HCT) levels >60%.

Adults with PK Deficiency are reported to have life-long hemolysis and the subsequent associated co-morbidities including anemia and transfusion dependence. Other co-morbidities include frequent miscarriages, aplastic crises, as well as symptoms associated with an acute on chronic hemolytic anemia (Rider, et al. 2011). Since unconjugated bilirubin is often chronically elevated, pigmented gallstones are common in children and adults. Severe and sometimes life-threatening iron deposition occurs and is typically progressive.

The current therapeutic options for patients with PK Deficiency are supportive. Most require life-long treatment, including blood transfusions at a frequency depending on the disease state. Long-term surveillance for systemic iron overload, even in transfusion-independent patients, is standard as is the use of chelation therapy. Case reports of cure by allogeneic bone marrow transplant have been published (Tanphaichitr, et al. 2000) but have been infrequently performed. Splenectomy, while not curative, can reduce the need for supportive blood transfusions and decrease bilirubin levels in some patients. However, this intervention is associated with significant morbidity including infection risk, and possible increased risk of thrombosis.

## 5.2. AG-348

AG-348 is an orally available, allosteric activator of PKR. It is hypothesized that drug intervention with AG-348 restores glycolytic pathway activity and normalizes red cell metabolism *in vivo*. Biochemical experiments demonstrate that AG-348 is a potent, pan-activator of many PKR alleles associated with PK Deficiency. PK Deficiency red cells and their progenitors are characterized by changes in metabolism associated with defective glycolysis, including a build-up of PEP and 2,3-diphosphoglycerate (2,3-DPG), and lowered ATP levels. Treatment of PK Deficiency patient red cells *ex vivo* with AG-348 results in increased ATP levels, and reductions in PEP and 2,3-DPG, consistent with pharmacological activation of PKR enzyme activity. *In vitro* studies against wild type (WT) PKR enzymes and *ex vivo* studies in blood from mice, monkeys and humans indicate that AG-348 activates WT PKR with a similar potency and efficacy as it does with the mutant versions. *In vivo* studies in mice confirm the potency of AG-348 in increasing WT PKR enzyme activity and modulating the levels of downstream markers such as ATP and 2,3-DPG. This therapeutic approach may be an effective way to correct the underlying pathology of PK Deficiency and, importantly, provide clinical benefit to patients.

### 5.2.1. Summary of Nonclinical Data

A series of exploratory pharmacology studies were conducted to characterize the ability of AG-348 to activate WT PKR and anemia-associated PKR mutants (mPKR) *in vitro*, *ex vivo*, and *in vivo*. Additional details regarding nonclinical data can be found in the AG-348 Investigator's Brochure

#### 5.2.1.1. Pharmacology

AG-348 is a potent, broad-spectrum activator of PKR with low nM potency against both WT and mutant enzymes. AG-348 is also able to effectively stabilize mPKR enzymes that are

hypersensitive to thermal denaturation, suggesting that AG-348 may be able to increase PKR pathway activity by preventing destabilization and degradation of mPKR enzymes. Its major metabolite, AGI-8702, has low potency mixed activity against PKR and mutant isoforms and can increase the thermostability of some mPKR, albeit with significantly weaker potency than that of AG-348.

The effect of AG-348 and AGI-8702 on PKR activity and a number of downstream pathway markers was evaluated in human RBCs. AG-348 dose-response curves in human whole blood showed potent activation of PKR, while AGI-8702 is a weak activator of the PKR enzyme. In whole blood from mice treated with AG-348, $^{13}$C-glucose labeling through the PKR reaction was increased by 80% demonstrating AG-348-mediated activation of the WT PKR enzyme. AG-348 treatment of RBCs from healthy human donors potently increased levels of the PKR reaction product ATP consistent with activation of PKR.

The effects of AG-348 on PKR activity and RBC metabolism also were assessed in blood samples from patients with PK Deficiency. AG-348 activated PKR and induced metabolic changes (increased ATP levels and decreased 2,3-DPG levels) consistent with increased glycolytic pathway activity in RBCs from PK Deficiency patients with different mutations in the PKR enzyme.

Assessments of binding inhibition potential and enzymatic activity were evaluated in a panel of 89 receptors, ion channels, and enzymes. Although AG-348 is a histamine H3 antagonist/inverse agonist and an aromatase inhibitor, no effects of histamine H3 modulation have been observed in safety pharmacology or toxicology studies. Effects consistent with aromatase inhibition were seen in toxicology studies (Section 5.2.1.4).

#### 5.2.1.2. Safety Pharmacology

Safety pharmacology studies demonstrated that neither AG-348 nor AGI-8702 exhibited significant inhibition of the human ether-à-go-go related gene (hERG) current (concentration of drug that achieved half-maximal inhibition [$IC_{50}$] 29.4 µM and > 10 µM, respectively). Additionally, in the monkey cardiovascular (CV) study, no CV effects were seen at any dose level. No effects were seen in AG-348 Good Laboratory Practice (GLP)-compliant Irwin and respiratory safety pharmacology studies in Sprague-Dawley rats. AG-348 administered via oral gavage had a dose-dependent emetic activity in the ferret at ≥ 30 mg/kg.

#### 5.2.1.3. Pharmacokinetics

Absorption, distribution, metabolism, and excretion (ADME) studies of AG-348 were performed in Sprague-Dawley rats, beagle dogs, and cynomolgus monkeys. Both oral (PO) and intravenous (IV) routes were evaluated at doses ranging from 1 to 50 mg/kg. A dose of 200 mg (50 mg/kg) was administered PO in the food-effect evaluation in monkeys. The PK of AG-348 in animal species is characterized by rapid oral absorption, medium to high total body plasma clearance (CLp), and high volume of distribution at steady-state ($V_{ss}$) in rats, dogs, and monkeys.

AG-348 has a medium to long apparent terminal half-life ($t_{1/2}$) in the rat, dog, and monkey. Medium to high oral bioavailability (F) was observed for AG-348 in animals. A high-fat diet led to lower oral absorption of AG-348 in monkeys.

AG-348 showed low brain penetration in rats (brain/plasma ratio of 5.7% to 9.1%) based on area under the plasma concentration versus time curve from 0 to 24 hours ($AUC_{0\ 24hr}$) following single and repeated-dose administration, respectively. Metabolism appears to be the major elimination pathway for AG-348 with minimal biliary or urinary excretion.

Both *in vitro* and *in vivo*, the predominant metabolite was the N-dealkylated product, AGI-8702. There were no metabolites unique to human liver microsomes and no direct Phase 2 metabolites. The plasma metabolite profile of AG-348 did not change with repeat or ascending dosing, suggesting that AG-348 is not susceptible to metabolic switching with increasing or prolonged dose administration.

AG-348 is a weak direct inhibitor of cytochrome P450 (CYP) 2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 (testosterone 6β-hydroxylation). There was no direct inhibition of CYP1A2, CYP2B6, or CYP3A4/5 (midazolam 1′-hydroxylation) by AG-348. There was evidence of metabolism-dependent inhibition of CYP2C19 (largely reversible) and CYP3A4 (largely irreversible) by AG-348. AG-348 is considered a weak time-dependent CYP3A4 inhibitor. AGI-8702 is a weak direct inhibitor of CYP2C9 and CYP2C19. AG-348 has the potential to cause an induction-related drug-drug interaction (DDI) with sensitive CYP2B6 and CYP3A4 substrates.

The routes of metabolism for AG-348 are via multiple CYPs with CYP3A4 contributing > 70% of the total. CYP1A2, CYP2C9, and CYP2C8 contribute approximately 6%, 10%, and 7% to the remaining metabolism of AG-348; other isoforms contribute < 4% each.

AG-348 is a substrate for P-glycoprotein (P-gp), but not breast cancer resistance protein (BCRP). AG-348 is an inhibitor of P-gp (91% and 99% inhibition at 41 and 411 μM, respectively), but does not inhibit BCRP. AGI-8702 is not an inhibitor of P-gp or BCRP under the concentrations tested (5 and 100 μM).

#### 5.2.1.4. Toxicology

In rats, the no-observed-effect level/no-observed-adverse-effect level (NOEL/NOAEL), determined as 2000 mg/kg, was associated with area under the plasma concentration versus time curve from 0 to 12 hours ($AUC_{0\text{-}12hr}$) values 223- to 526-fold the projected human efficacious $AUC_{0\text{-}12hr}$ value. In dogs, clinical observations consistent with anaphylactoid reactions were seen, and the maximum tolerated dose (MTD) was 62.5 mg/kg, which was associated with an $AUC_{0\text{-}12hr}$ value 5.7-fold the projected efficacious $AUC_{0\text{-}12hr}$ value. The NOEL/NOAEL in dogs was 10 mg/kg, which was associated with an $AUC_{0\text{-}12hr}$ value 0.8-fold the projected efficacious $AUC_{0\text{-}12hr}$ value. In monkeys, the NOAEL was 1000 mg/kg, with only non-adverse emesis and body weight loss seen; this dose was associated with an $AUC_{0\text{-}12hr}$ value 70-fold the projected efficacious $AUC_{0\ 12hr}$ value. Based on the results of single-dose studies, the rat and the monkey were chosen as the most appropriate species for further evaluation in toxicology studies.

Dose-limiting toxicity (DLT) in cynomolgus monkeys was defined in non-GLP 5-day and 14-day repeat-dose studies as emesis, inappetence, and weight loss. These toxicities became dose limiting at $AUC_{0\text{-}12hr}$ values 27- to 34-fold the projected efficacious $AUC_{0\text{-}12hr}$ value, and precluded meaningful evaluation of other toxicities at this exposure level. Potential other effects at this exposure level were observed in a number hematology and serum chemistry parameters as well as in lymphoid organs. Additionally, minimal potential effects in kidneys (renal

tubulointerstitial nephritis) and heart (myocardial degeneration), which could not be differentiated from spontaneous background lesions, were seen when monkeys were exposed to AG-348 $AUC_{0\text{-}12hr}$ values ≥ 27- to 34-fold the projected human efficacious $AUC_{0\text{-}12hr}$ value.

In GLP-compliant 28-day monkey study, the dose of 150 mg/kg/day (75 mg/kg/dose twice daily [BID]) was the NOAEL. Effects were limited to increased liver weights without serum chemistry or microscopic correlate. At this dosage level, the Day 27 $AUC_{0\text{-}12hr}$ values were 8.9- and 8.5-fold the projected efficacious $AUC_{0\text{-}12hr}$ value in males and females respectively. In the same study, the low dosage of 20 mg/kg/day (10 mg/kg/dose) resulted in $AUC_{0\text{-}12hr}$ values that approximated the efficacious $AUC_{0\text{-}12hr}$ value, and there were no test article-related effects seen. The next highest dose of 50 mg/kg/day (25 mg/kg/dose) was the NOEL and was associated with $AUC_{0\text{-}12hr}$ values 3.1- and 2.6-fold the projected efficacious $AUC_{0\text{-}12hr}$ value in males and females respectively.

In the GLP-compliant 28-day rat study, the NOAEL in males was the highest dose tested, 600 mg/kg/day (300 mg/kg/dose), and the NOAEL in females was the lowest dose tested, 20 mg/kg/day (10 mg/kg/dose). At the 600 mg/kg/day dosage level in males, AG-348-related findings were limited to mild effects on hematology, serum chemistry, and urinalysis parameters, and microscopic findings in the adrenal gland (minimal to mild vacuolation of the adrenal zona glomerulosa and decreased thickness of the zona fasciculate), liver (minimal to mild hepatocellular hypertrophy), kidney (minimal tubular vacuolation), pancreas (minimal to moderate decreased zymogen granules), heart (minimal myocardial vacuolation), and prostate (minimal to mild decreased secretion). All findings were fully reversible over the 14-day recovery period with the exception of decreased serum glucose levels and decreased prostate secretion. In females, the highest dosage tested was 200 mg/kg/day (100 mg/kg/dose); adverse effects observed were similar to those observed in the 600 mg/kg/day males, with the exception that in females, fewer effects in hematology and serum chemistry parameters were seen, and also in females, adverse effects in the reproductive organs consistent with aromatase inhibition were observed.

In the 13-week repeat-dose rat study, adverse effects in males were identified in the testes and consisted of seminiferous tubular degeneration, spermatid retention, and Leydig cell hypertrophy. These effects were defined as adverse at dose levels at which they are expected to impair fertility. These effects were either not present or present with incidence and severity similar to that of the vehicle group in lower dose levels. Adverse effects in females included uterine atrophy and increased folding of the luminal surface; these effects were defined as adverse at the dose level at which they are expected to impair fertility

In the 13-week repeat-dose study in monkeys, no adverse effects were identified, and no new effects were identified when compared to the 4-week repeat-dose study. Similar to what occurred on the 4-week study, inappetence and emesis during the initial 1-2 weeks of dosing occurred, precluding evaluation of higher doses.

AG-348 inhibits human aromatase activity with an $IC_{50}$ of 2050 nM (based on human placental microsomes) and rat aromatase with an $IC_{50}$ of 493 nM (based on rat ovarian microsomes). In the GLP-compliant 28-day rat study, histologic effects consistent with aromatase inhibition were seen in the female reproductive tract at the mid- and high-dosage levels (100 and 200 mg/kg/day) and included incomplete corpora lutea; ovarian follicular cysts; ovarian cystic, luteinized follicles; uterine atrophy; vaginal mucification; and altered cyclicity. Although these findings

were minimal to mild and were fully reversible (over 14 days), they were considered adverse and the next lower dosage evaluated, 20 mg/kg/day (10 mg/kg/dose BID) was the NOAEL in females. The Day 27 $AUC_{0\text{-}12hr}$ value associated with this dosage level was 6.9-fold the projected human efficacious $AUC_{0\text{-}12hr}$ value. The potential for aromatase inhibition effects occurring in female rats at $AUC_{0\text{-}12hr}$ values > 6.9-fold and < 53-fold the projected efficacious $AUC_{0\text{-}12hr}$ value has been addressed in a 13-week rat study. In this study using doses between the NOAEL and LOAEL in the 28-day study, the NOAEL for histologic lesions of the uterus that may be associated with aromatase inhibition resulted in an $AUC_{0\text{-}12hr}$ that was 26-fold the projected efficacious value. Notably, due to the potency difference of AG-348 against rat versus human aromatase inhibition, there is potential for a 4-fold wider margin for aromatase inhibition in humans versus rats. AGI-8702 is not an aromatase inhibitor.

### 5.2.2. Summary of Clinical Data

To date, 72 healthy adult volunteers have been exposed to AG-348 in 2 clinical studies, a single ascending dose (SAD) study and a multiple ascending dose (MAD) study, with 31 of these subjects exposed to a single AG-348 dose under fasted conditions; 5 exposed to 2 doses of AG-348 under fasted and fed conditions; and 36 exposed to repeated doses of AG-348 for up to 14 days[1]. The following discussion of clinical data refers only to healthy adult volunteer subjects, as this is the first clinical trial in which patients with PK Deficiency will be treated with AG-348.

#### 5.2.2.1. Pharmacokinetics

The PK of AG-348 showed low to moderate variability between subjects. After single doses of AG-348 from 30 to 2500 mg, AG-348 was rapidly absorbed as indicated by the short time to maximum plasma concentration ($T_{max}$). There appeared to be some lengthening of the period of absorption at higher doses as indicated by higher $T_{max}$ and a less than proportional increase in maximum concentration ($C_{max}$). Dose-normalized area under the curve (AUC) generally remained constant over the dose range studied, suggesting that AG-348 total exposure increased in a dose-proportional manner. The mean $t_{1/2}$ ranged from 17.8 to 20.4 hours when samples were collected through 72 hours and from 50.3 to 79.3 when samples were collected through 120 hours. However, this terminal elimination phase contributed little to overall exposure of AG-348, as indicated by the small difference between $AUC_{0\text{-}12}$ and area under the plasma concentration versus time curve from 0 to infinity ($AUC_{0\text{-}\infty}$), suggesting a shorter effective half-life of approximately 3 to 6 hours. AG-348 was extensively distributed (mean apparent volume of distribution [$V_z/F$] range of 271 to 1148 L) and had a moderate rate of clearance (geometric mean clearance [CL/F] range of 10.3 to 13.9 L/hr). The fraction of AG-348 excreted in urine ranged from 0.0145 to 0.0209 across the dose levels suggesting that renal excretion plays a minor role in the systemic elimination of AG-348.

The preliminary repeat-dose PK of AG-348 at doses ranging from 15 mg every 12 hours (q12h) to 700 mg q12h also showed an increase in AG-348 exposure in a dose-proportional manner after the first dose. AG-348 exposure observed after repeated dosing for 14 days at doses of

[1] At the time of this document, results from ongoing Study AG348-C-002 in healthy volunteers are blinded. Based on the randomization scheme, 36 subjects were assigned to AG-348 and 12 subjects were assigned to placebo.

120 mg every 24 hours (q24h) to 700 mg q12h were lower than observed on Day 1 after the first dose. The magnitude of the effect was greater at higher doses; lower doses of 15 mg q12h and 60 mg q12h did not show this effect. This observation may be related to the nonclinical finding that AG-348 is a potential inducer of human CYP3A4, an enzyme which biotransforms AG-348. It is possible that multiple doses of AG-348 leads to an increased rate of its own metabolism.

Evaluation of the effect of food on the PK of a single 700 mg dose of AG-348 in 5 subjects who were administered the drug fasting and then, after an appropriate wash-out period, re-administered the drug following ingestion of a standard US Food and Drug Administration (FDA) high fat meal, showed that food likely has a minimal effect on the PK of AG-348.

#### 5.2.2.2. Pharmacodynamics

After a single dose of AG-348, a decrease in the concentration of 2,3-DPG was observed at 3 hours post-dose, decreased in a dose-dependent manner to a minimum at 24-hour post-dose, and then returned to values similar to baseline by 72 to 120 hours post-dose. The mean decrease at 24 hours was approximately 300 µg/mL at the 700 through 2500 mg dose levels. Similar decreases were observed after the first dose of multiple doses and prior to doses in the midst of the MAD study. After the final dose of multiple doses, the concentration of 2,3-DPG returned to values similar to baseline between 72 and 120 hours post- dose.

After a single dose of AG-348, a minimal increase in the concentration of ATP was observed at 24 to 120 hours post-dose. In the MAD study, no increase was observed at 12 hours after the first dose; the concentration of ATP was increased on Day 8 to concentrations greater than in the single dose study, continued to trend upward on Day 11, and remained at a similar level through Day 14. The concentration of ATP remained elevated through 120 hours after the last dose on Day 14. The magnitude of the increase in ATP was similar across the dose range from 60 mg BID to 700 mg BID.

#### 5.2.2.3. Safety

Overall, AG-348 has been well-tolerated among healthy volunteers.

After a single AG-348 dose, treatment-emergent adverse events (TEAEs) reported by > 1 subject at any time on study (either under fasted or fed conditions) included nausea (14%), headache (14%), and upper respiratory tract infection and vomiting (each 6%). After repeated dosing of AG-348 for 14 days, based on blinded data, TEAEs that occurred in > 2 subjects within any cohort and therefore must have been experienced by at least 1 AG-348-treated subject included dizziness, feeling hot, headache, hyperhidrosis, nausea, restlessness, and vomiting.

A dose-relationship was apparent with regard to the incidence of gastrointestinal events, primarily nausea and vomiting, with the incidence of such events increasing with increasing dose. Nausea and/or vomiting were observed only at doses ≥ 1400 mg in the single dose study and only at doses ≥ 700 BID in the MAD study. Nausea and vomiting were not observed at any dose ≤ 360 mg in either the single or multiple dose studies.

All but 1 TEAE reported to date has been mild or moderate (Grade 1 or 2) in intensity. The only Grade 3 TEAE was elevated liver transaminases (alanine aminotransferase [ALT]; aspartate aminotransferase [AST]) in a single subject in the MAD study treated with 700 mg of blinded study drug q12h BID. The treatment this subject received (AG-348 *versus* placebo) remains

blinded, and the events were considered to be study drug-related and led to study drug discontinuation, following which the elevated liver transaminases resolved. No subject discontinued in the SAD study due to an adverse event (AE). Four subjects, at doses of 700 mg BID, have discontinued study drug in the MAD study due to at least 1 TEAE, with nausea and vomiting contributing to study drug discontinuation in 3 of these 4 subjects. The fourth subject discontinued for a cutaneous rash.

No deaths or other serious adverse events (SAEs) have been reported in any clinical study of AG-348. Furthermore, no DLTs have been declared after a single administration of AG-348 at doses of 30 mg to 2500 mg or after repeat administration of AG-348 for 14 days at daily doses of 120 mg to 1400 mg (although the event of Grade 3 elevated liver function tests described above in the MAD study may be assessed as DLT after the subject's treatment assignment is unblinded).

Due to preclinical observations pertaining to the potential for inhibition of the aromatase enzyme (see Section 5.2.1.4), the AG348-C-002 study included assessment of baseline and serial measures of free and total serum testosterone and serum estradiol and estrone. The data are still blinded, and therefore, no quantitative statistical analyses have yet been performed. Upon qualitative review of the serum hormone data, at least some subjects in the 120 mg BID cohort and higher dose cohorts demonstrate modest increases in androgenic hormones and decreases in estrogens, compatible with a potential signal of aromatase inhibition. Additional analyses will be performed after the study is completed and the data are locked and unblinded.

## 5.3. Study Rationale

Study AG348-C-003 is the first study that will be conducted in patients with PK Deficiency. This study is primarily intended to evaluate the safety and tolerability and potential indicators of clinical activity of AG-348 administered for up to 24 weeks. This study will also evaluate the PK profile of AG-348 and its metabolite AGI-8702, the PD responses in ATP and 2,3-DPG following administration of AG-348, and the clinical activity of AG-348 in PK Deficiency patients. Two previous double-blind, placebo-controlled clinical trials of AG-348 conducted in healthy adult male and female volunteers (AG348-C-001, a SAD study; and AG348-C-002, a MAD study) have established an acceptable safety and tolerability profile for AG-348 for up to 14 days of both once-daily (QD) and BID dosing at exposures that result in significant pharmacodynamic (PD) changes in whole blood levels of the glycolytic metabolites 2,3-DPG and ATP. The target population of this study consists of adult males and females with a diagnosis of PK Deficiency, who are anemic but non-transfusion dependent. Non-transfusion dependent patients are preferred for this study in order to reduce any potential confounding effect of transfusion therapies on evaluation of potential indicators of clinical activity and PD responses. The safety, tolerability, and PK/PD findings in this study will form the basis for subsequent clinical development of AG-348.

### 5.3.1. Summary of Overall Safety Management Plan

Measures to minimize the risks to patients enrolled in this study have been taken with respect to the following study design elements:

- The initial doses for Arms 1 and 2 have been selected on the basis of safety and tolerability observations already carefully made in two predecessor clinical trials in adult healthy male and female volunteers;
- The specified inclusion/exclusion criteria have been carefully considered to avoid enrollment of subjects for whom exposure to the study drug might pose a hazard;
- A designated Data Review Team (DRT) consisting of the overall study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer has been established to meet regularly at approximately 6-week intervals throughout the duration of the study to review the accumulating study data and will exercise options to suspend enrollment to one or both of the initial two study dose arms, discontinue enrollment to one or both of the initial two study dose arms, adjust the dose of patients in one or both of the initial two study arms, and/or implement one new study dose arm. If one new dosing arm is implemented, the dose selected will not exceed 360 mg BID, the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy volunteers. Group cohort stopping rules for terminating enrollment into an arm based on the severity (CTCAEv4.03 grade) and frequency of AEs are defined;
- Dose modification and stopping rules are defined for individual patients;
- Guidance for permitted, prohibited, and cautionary concomitant medications is specified based on the estimated potential for drug-drug interactions from hepatic cytochrome enzyme interactions with AG-348.

# 6. TRIAL OBJECTIVES AND ENDPOINTS

## 6.1. Primary Objective

The primary objective of the study is to:

- Evaluate the safety and tolerability of up to 24 weeks of AG-348 administration in patients with PK Deficiency.

## 6.2. Secondary Objectives

The secondary objectives of the study are to:

- Evaluate the pharmacokinetics (PK) of AG-348 and the metabolite AGI-8702.
- Evaluate the PD response of ATP and 2,3-DPG after administration of AG-348.
- Evaluate indicators of clinical activity of AG-348 in patients with PK Deficiency, including changes in hemoglobin (Hb), HCT, reticulocyte count, haptoglobin (Hp), carboxyhemoglobin (COHb), lactate dehydrogenase (LDH), total and indirect bilirubin, erythropoietin (EPO), ferritin, and transferrin saturation (serum iron/iron binding capacity).

## 6.4. Study Measures and Endpoints

### 6.4.1. Safety Measures and Endpoints

Safety will be evaluated by:

- Monitoring of AEs, including determination of SAEs and AEs leading to discontinuation; safety laboratory parameters (hematology, chemistry, urinalysis, coagulation); physical examination findings; vital signs (VS); and 12 lead electrocardiograms (ECGs). Adverse events will be graded using Common Toxicity Criteria for Adverse Events (CTCAE), Version 4.03. Serum sex hormone levels (testosterone, estrone, and estradiol), bone turnover markers (serum N-terminal telopeptide [NTX] and serum C-terminal telopeptide [CTX]), total cholesterol, high-density lipoprotein-C (HDL-C), and triglycerides will be monitored for evidence of potential inhibition of aromatase by AG-348.

### 6.4.2. Clinical Activity Measures and Endpoints

- Monitoring of potential indicators of clinical activity will include evaluating changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation.

### 6.4.3. Pharmacokinetic and Pharmacodynamic Measures and Endpoints

The PK and PD profile of AG-348 will be evaluated by:

- Approximately the first 10 patients treated, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 15.1, Table 5. The remainder of treated patients will undergo limited PK sampling as detailed in Appendix 15.1, Table 6. Serial blood sampling for determination of concentration-time profiles of AG-348 and its metabolite AGI-8702 will be conducted following the first dose and the morning Day 15 dose, and additional trough levels of AG-348 and AGI-8702 will be obtained. AG-348 and AGI-8702 will be analyzed using qualified assays to determine concentrations in plasma. Pharmacokinetic parameters on Day 1 and Day 15 will be computed using standard non-compartmental methods based on observed plasma AG-348 and AGI-8702 concentrations.
- Pharmacodynamic assessments will include 2,3-DPG, ATP (secondary objectives), [redacted] Approximately the first 10 patients treated will undergo extensive PD sampling as detailed in Appendix 15.1, Table 5. The remainder of treated patients will undergo limited PD sampling as detailed in Appendix 15.1, Table 6. Serial blood sampling for determination of levels of ATP and, 2,3-DPG will be conducted following the first dose and the morning Day15 dose, and additional trough levels of ATP and 2,3-DPG will be obtained. ATP and 2,3 DPG will be analyzed using qualified assays to determine concentrations in whole blood. PD parameters on Day 1 and Day 15 will be computed based on observed whole blood ATP and 2,3-DPG concentrations.

[redacted]

# 7. INVESTIGATIONAL PLAN

## 7.1. Overall Study Design

Study AG348-C-003 is a Phase 2, open-label, two-arm, multicenter, randomized, dose-ranging study during which adult patients with PK Deficiency will receive multiple doses of AG-348 for up to 24 weeks. Patients with PK Deficiency confirmed by red blood cell PK enzymatic assay performed at Screening will be eligible to participate in this study. At Week 25, patients who safely tolerate AG-348 and demonstrate clinical activity of AG-348 may be eligible to immediately roll over to a separate safety extension study for continued treatment. Patients who finish treatment at the end of 24 weeks or sooner will undergo follow-up assessment 4 weeks after the last dose of study drug. Patients with toxicity suspected to be related to study drug will continue follow-up until the AE resolves, is declared chronic by the Investigator, or the patient is lost to follow-up.

Initially, up to 25 patients will be randomized on an open-label, 1:1 basis to each of two BID doses of AG-348 (up to 50 patients; see Figure 3, Study Schema). The dose of Arm 1 is 300 mg of AG-348 administered orally q12h (BID). The dose of Arm 2 is 50 mg of AG-348 administered orally q12h (BID) for 12 weeks. Randomization will be stratified by *PKR* mutation in order to maintain balance as much as possible across the dose arms for the specific mutations expected to be most frequently enrolled. The *PKR* mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H) and all other mutations ("other"). Mutation status is defined by the presence of at least one of the indicated mutations; patients with more than one stratified mutation will be assigned based on Sponsor's discretion.

The doses for each arm have been selected from the forerunner AG348-C-001 SAD study and AG348-C-002 MAD studies in healthy adult volunteers to represent the range of doses/exposures that were safely tolerated and resulted in maximal or near maximal PD effects on 2,3-DPG and ATP.

Because PK Deficiency is a rare disease with a limited eligible patient population and because the underlying pathophysiology and clinical phenotype of affected patients is heterogenous due to the wide variety of mutations in PKR that cause the disease, it is deemed important to focus closely on dose findings in this first-in-patient study. Therefore, in addition to initiating this study with 2 different doses of AG-348 administered q12h, a DRT will be established to review study data on a frequent basis and adapt the study design, dose and schedule of AG-348 as indicated.

The DRT will monitor safety on an on-going basis and meet at regular intervals (approximately every 6 weeks), or *ad hoc* as necessary, to review AEs, VS, clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and ECGs on enrolled patients. The DRT will also review available PK/PD data and indicators of clinical activity (e.g., changes from baseline in Hb). The DRT will be comprised of the study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer.

Approximately every 6 weeks, beginning 6 weeks after the first patient is doses or *ad* hoc as necessary, the DRT will review cumulative safety data, available PK/PD data, and clinical

activity data. Based on the DRT's recurring 6-week reviews, the DRT may exercise one or more of the following options:

- Continue treatment and enrollment in existing arms without change.
- Add 1 new dose arm (Arm 3) to enroll up to 25 patients at a dose to be determined; the dose for Arm 3 may be lower or higher than Arm 1 and Arm 2 doses, but will not exceed 360 mg q12h; and the dose regimen may be less frequent than q12h.
- Terminate or suspend enrollment to allow further review of clinical data in Arm 1 and/or Arm 2 (and/or potential Arm 3). Enrollment in an arm could be terminated or suspended to allow further review, for example, for unacceptable safety/tolerability, poor PD response, or lack of signs of clinical activity.
- Re-assign patient's doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the patients in the terminated arm will remain in their original arm, i.e., they will not count towards the enrollment quota of the arm whose dose and schedule is being adopted.
- Implement specific genotype restrictions to enrollment in one or more arms to ensure representation of patients with genotypes of greatest clinical relevance.

The DRT will perform these evaluations on a recurring 6-week basis. The data that the DRT will review to make these decisions is expected to include, but are not necessarily limited to, the following:

- *Safety Observations:* all AEs; VS, clinical laboratory (hematology, clinical chemistry, coagulation, and urinalysis), and ECGs;
- *PK and PD Observations:* including changes in 2,3-DPG and ATP;
- *Indicators of Clinical Activity:* including changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, EPO, total and indirect bilirubin, ferritin, and transferrin saturation.

If a third dose arm is implemented, the dose of AG-348 selected will not exceed 360 mg BID, as this was the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy volunteers. The PK/PD sampling schedule in a potential third dosing arm will be determined by Sponsor's discretion and may follow either the extensive or limited PK/PD sampling schedules as specified.

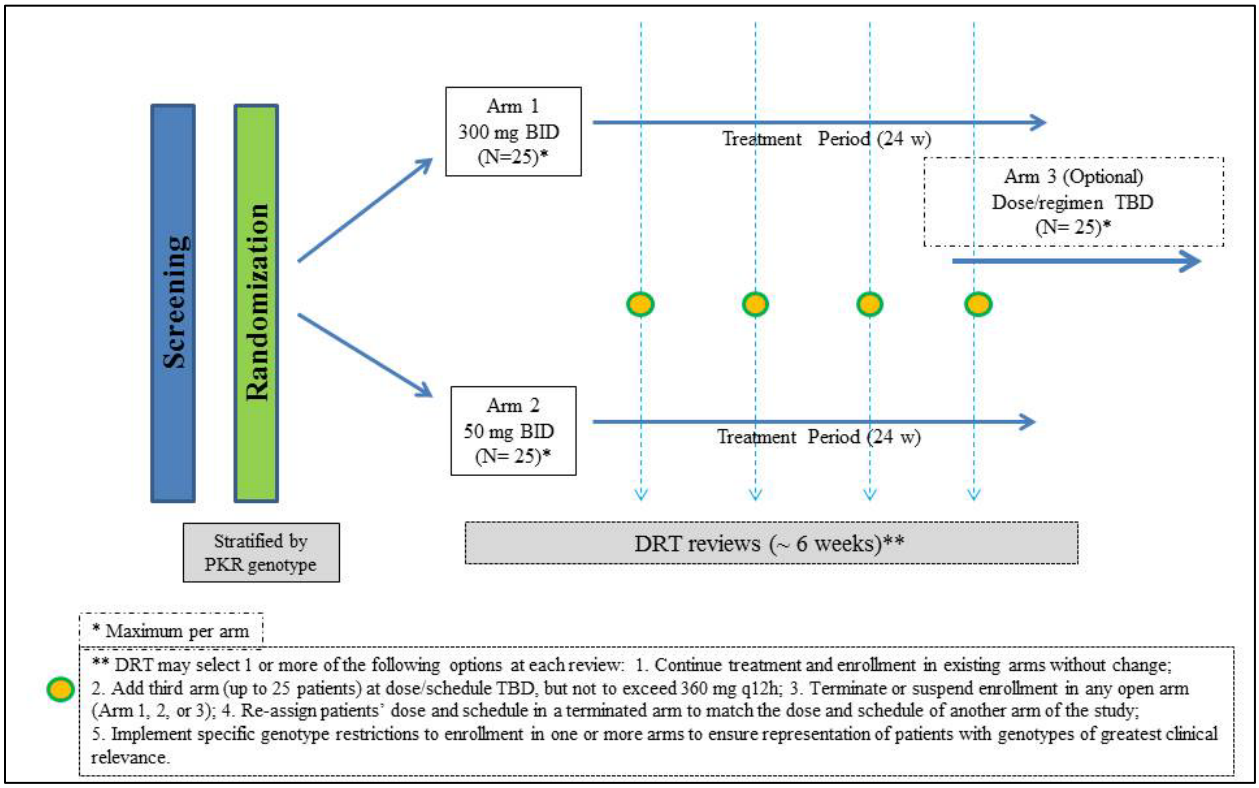
The DRT will monitor the safety data in an ongoing manner as described and may make a decision to terminate enrollment in an arm if unacceptable AEs are observed, PD activity is limited, and/or markers of clinical activity indicate no effect. Unacceptable AEs are defined as ≥ Grade 3 AEs (using National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAEv4.03]) that are assessed as at least possibly related to AG-348, with the exception of Grade 3 headache, hot flash/flushing, nausea, vomiting, and/or diarrhea that is transient in duration (< 24 hours) or able to be medically managed to ≤ Grade 2 within 24 hours. The DRT may also exercise discretion to terminate enrollment in an arm if it observes a frequency of Grade 2 AEs that would make it difficult to support long-term dosing.

As the number of enrolling arms changes in the study (for example, from 2 to 3), the randomization scheme will adjust to enable balanced randomization into each actively accruing

arm. Randomization and stratification will cease in the event that only a single arm is left enrolling.

Depending on possible early termination of 1 or both of the initial 2 arms, or the addition of a third arm, the study could enroll up to a maximum of 75 patients.

**Figure 3: Study Schema**




Abbreviations: BID (q12h) = twice-daily (every 12 hours); DRT = data review team; PKR = pyruvate kinase red blood cell isoform; TBD = to be determined; w = weeks.

The Investigator will monitor all patients for safety and tolerability. Modification of an individual patient's dose of AG-348 will be based on AEs and observed changes in Hb levels as detailed in Section 9.7.1 and Section 9.7.2.

Screening assessments will occur within 28 days prior to the first dose of study treatment. During the Treatment period, patients will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12), and monthly through Week 24 (Weeks 16, 20, and 24). Patients who safely tolerate AG 348 and demonstrate evidence of clinical activity of AG-348 through Week 24 may be eligible to immediately enter a separate extension study for continued treatment. For patients who finish treatment at the end of 24 weeks or sooner, or who elect not to enter the extension trial, study discharge will occur 4 weeks (Week 28 or earlier) following the last dose of study treatment at the final follow-up assessment.

Safety assessments will include monitoring of AEs, including determination of SAEs and AEs leading to discontinuation; safety laboratory parameters (e.g., hematology, serum chemistry, coagulation studies, and urinalysis); physical examination findings; VS; and 12 lead ECGs.

Additional safety assessments will include monitoring of sex hormone levels (testosterone [total and free], estrone, and estradiol), and bone turnover markers (NTX and CTX). Follow-up assessments will be conducted on Day 197 (Week 28) and will include physical examination, weight, performance status, VS, 12-lead ECGs, laboratory evaluations (hematology, Hp, EPO levels, serum chemistry, coagulation studies, urinalysis; lipids, hormonal testing), AEs, and transfusion record.

Pharmacokinetic assessments will include serial blood sampling for PK profiles of AG-348 and its metabolite AGI-8702. Pharmacodynamic evaluations will include serial blood sampling for determination of levels of ATP and 2,3 DPG. Extensive PK/PD sampling will be conducted on the first approximately 10 patients total treated in Arms 1 and 2 (see Appendix 15.1, Table 5) while more limited PK/PD sampling will be conducted on additional patients treated if enrollment in Arm 1 or Arm 2 is expanded or if an alternate dosing arm is added (see Appendix 15.1, Table 6).

## 7.2. Justification of the Study Design

The primary and secondary objectives of this study are to evaluate the safety, tolerability, PK and PD, and indicators of clinical activity of AG-348 in patients with PK Deficiency. The choice of dose and schedule of administration of AG -348 for Arms 1 and 2 was based on the highest safely tolerated dose (Arm 1: 300 mg BID) and the lowest dose with potentially relevant PD activity (Arm 2: 50 mg BID) from the forerunner AG348-C-002 MAD study in healthy volunteers. Decisions regarding continuing enrollment and treatment in these initial dose arms and/or implementation of an additional dose arm will be based on DRT review of safety, PK and PD data, and indicators of clinical activity collected from all patients treated in Arm 1 and Arm 2. This design was chosen to minimize risk to patients while allowing evaluation of safe and pharmacologically active dose levels of AG-348, and to allow the necessary flexibility to adjust dose and schedule should the safety, tolerability, PK, and/or PD be different in patients with PK Deficiency compared with healthy volunteers.

Additional safety measures intended to minimize risk to patients include monitoring of AEs by the DRT and specified provisions for individual patient dose modification as needed for safety and (potentially) large increases in Hb level (Section 9.7.1 and Section 9.7.2). Measures intended to maximize the opportunity for patients with demonstrated safety and tolerability to continue to derive benefit from any observed clinical activity of AG-348 include the option for continued treatment in a separate extension study.

A comprehensive series of safety evaluations, including laboratory parameters, physical examinations, VS, 12-lead ECGs, and monitoring for SAEs and AEs, will be conducted to evaluate the safety profile of AG-348 and to aid in the determination of the recommended dose for continued development.

The study includes serial blood sampling across at least two different doses of the study drug to assess its PK and PD profiles.

Consistent with the design of many Phase 2 studies, preliminary evaluation of the potential PD and clinical activity of AG-348 are secondary objectives of this study. The latter will include assessments of the PK/PD relationship between AG-348 and the biomarkers ATP and 2,3-DPG, [redacted]

## 7.3. Rationale for the Starting Dose and Dose Range

Prior to execution of this study, Agios conducted two clinical studies of AG-348 in healthy volunteers, including a SAD study (AG48-C-001) and a MAD (14 day q12h) study (AG348-C-002). Available details of these studies are discussed in the current Investigator's Brochure (IB). Between these two studies, 72 healthy human subjects have been dosed with AG-348. *In vitro* investigations, also reported in the IB, had previously demonstrated that AG-348 increased the activity of wild-type PKR approximately to the same extent as it did a series a recombinant mPKRs. Therefore it was deemed reasonable to study the safety, tolerability, PK, and PD of AG-348 in healthy subjects in a controlled Phase 1 setting as a more efficient means of obtaining information than in the actual rare disease population of patients with PK Deficiency.

The MAD study demonstrated that the exposures produced by AG-348 doses from 60 mg q12h to 360 mg q12h (including 120 mg q24h) resulted in maximal changes from baseline for the PD markers 2,3-DPG (reduction from baseline) and ATP (increase from baseline). The exposures resulting from doses less than 60 mg q12h were of lesser magnitude and the exposures resulting from doses greater than 360 mg q12h were of no greater magnitude than the aforementioned range. Therefore the starting doses for this first dose ranging study in patients with PK Deficiency were selected to be 300 mg q12h (Arm 1) and 50 mg q12h (Arm 2). These doses were demonstrated to be safe and tolerable in the healthy volunteer studies. The availability of ATP is proposed as being critical for optimally maintaining RBC membrane integrity (see Section 5.1). The dose ranges from 50 mg q12h to 300 mg q12h may result in clinically effective modulation of PKR in PK Deficiency patients if the mutated enzyme is responsive to AG-348 in a similar manner to the wild-type enzyme in healthy subjects. However, there are many different mutations in PKR that result in PK Deficiency, and these mutations produce variable effects on the enzyme in terms of catalytic activity and thermal stability. It is not known if different mutations will respond clinically in a similar manner to the same exposure to AG-348. Therefore, it is prudent to study the range of safe and pharmacodynamically relevant doses as specified in this study, and to allow flexibility for the DRT to analyze the evolving study data to adapt the dose and schedule of administration of AG-348 to produce the optimal combination of safety, tolerability, and PD, and, potentially, clinical response.

## 7.4. Criteria for Study Termination

This study may be prematurely terminated if, in the opinion of the Sponsor, there is sufficient reasonable cause. In the event of such action, written notification documenting the reason for study termination will be provided to each Investigator.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to patients;
- Insufficient adherence to protocol requirements;
- Plans to modify, suspend, or discontinue the development of the study drug;
- Other administrative reasons.

Should the study be closed prematurely, all study materials must be returned to the Sponsor or the Sponsor's designee.

# 8. STUDY POPULATION

## 8.1. Number of Patients

Up to approximately 75 patients may be enrolled in this study.

## 8.2. Inclusion Criteria

For entry into the study, patients must meet all of the following criteria during the Screening or other specified period:

1. Signed written informed consent obtained prior to performing any study procedure, including screening procedures.
2. Male or female, aged 18 years and older.
3. Known medical history of PK Deficiency.
4. All patients must have documented clinical laboratory confirmation of PKD by RBC pyruvate kinase enzymatic assay performed at Screening by a designated central laboratory. Patients with prior documentation of PK Deficiency by RBC enzymatic assay will have a reconfirmation of this result during Screening as a condition of enrollment.
    a. In the event that a patient's screening pyruvate kinase enzymatic assay is negative (i.e., shows normal pyruvate kinase activity), the patient will be eligible for enrollment if the genotyping shows a mutant genotype that has been previously documented in the literature to be associated with pyruvate kinase deficiency. If the genotyping shows a previously undescribed mutation in the PKR gene, then the eligibility for enrollment will be determined on a case-by-case basis by the Coordinating Investigator and Medical Monitor in discussion with the Investigator. If no mutation is defined, then the patient will not be eligible.
5. All patients must have genotypic characterization of the mutant PKR gene performed by a designated central laboratory at Screening, unless genotype is available from the patient's participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480).
6. All patients must have genotypic characterization of the UGT1A1 gene performed by a designated central laboratory to document whether they may have underlying Gilbert's Disease. Patients with Gilbert's Disease are eligible to enroll.
7. Males must have Hb ≤ 12.0 g/dL; females must have Hb ≤ 11.0 g/dL.
8. All patients must be considered transfusion independent as defined by: no greater than 3 units of RBCs transfused in the 12-month period up to the first day of study dosing and no transfusions within 4 months of first day of study dosing. Patients who have received more transfusion support than described above will evaluated for eligibility on a case-by-case basis by the Medical Monitor.
9. Splenectomized patients:
    a. Must have undergone their procedure at least 6 months prior to Screening.

b. Must be current in their vaccinations for Pneumococcal Conjugate (PCV13), Pneumococcal Polysaccharide (PPSV23), Quadrivalent Meningococcal vaccine, and Haemophilus influenzae type b (Hib) as recommended by Centers for Disease Control and Prevention's (CDC) Advisory Committee on Immunization Practices (ACIP) or immunization advisory groups in Canada and the European Union (for patients enrolled in Canada and the EU). [http://www.cdc.gov/vaccines/schedules/downloads/adult/adult-schedule.pdf] [Any missing vaccinations may be administered during the screening period.]

10. Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 2.
11. Patients must be taking at least 1 mg of folic acid daily for at least 21 days prior to first dose and continued daily during study participation.
12. Adequate organ function, defined as:
    a. Serum AST and ALT ≤ 1.5 × upper limit of normal (ULN) (unless the increased AST is assessed by the Investigator as due to hemolysis).
    b. Normal or elevated levels of serum bilirubin. In patients with serum bilirubin > ULN, the elevation must be attributed to hemolysis with or without Gilbert's syndrome and must not be choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease.
    c. Serum creatinine ≤ 1.25 × ULN. If serum creatinine > 1.25 × ULN, then 24-hour measured or calculated (Cockcroft-Gault) glomerular filtration rate (GFR) ≥ 60 mL/min.
    d. Absolute neutrophil count (ANC) > 1.0 × 109/L.
    e. Platelet count ≥ 100 × 109/L.
    f. Activated partial thromboplastin time (aPTT) and international normalized ratio (INR) ≤ 1.25 × ULN, unless the patient is receiving therapeutic anticoagulants.
13. Women of childbearing potential (WOCBP) must agree to abstain from sexual intercourse or to use an acceptable/effective method of contraception (i.e., condom plus spermicide, condom plus oral contraceptive, condom plus intrauterine device [IUD], condom plus diaphragm with spermicide) from as soon as feasible during the Screening period until 30 days following the last dose of AG-348.
    a. WOCBP include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, and/or bilateral oophorectomy) or is not post-menopausal. Post-menopausal is defined as:
        i. Amenorrhea ≥ 12 consecutive months without another cause, and a documented serum follicle stimulating hormone (FSH) level > 35 mIU/mL;
        ii. Amenorrhea≥ 12 consecutive months in women ≥ 62 years old (FSH testing is not required).

14. WOCBP must have a negative serum or urine pregnancy test within 72 hours before start of AG-348 dosing.
15. Women must not be breastfeeding.
16. Male patients, with the exception of those who have undergone vasectomy at least 6 months prior to Screening, must agree to abstain from sexual intercourse or, if sexually active, to use a condom with spermicide as contraception (regardless of their female partner's childbearing potential or their partner's use of their own contraception) from Day 1 of dosing until 30 days following the last dose of AG-348.

## 8.3. Exclusion Criteria

Patients who meet any of the following criteria at Screening or prior to dosing on Day 1 will not be enrolled in the study:

1. Hemoglobin level > 12.0 g/dL if male; Hb > 11.0 g/dL if female.
2. Additional diagnosis of any other congenital or acquired blood disorder, including glucose-6-phosphate-dehydrogenase (G6PD) deficiency, or any other hemolytic anemia process except for mild allo-immunization as a consequence of transfusion therapy.
3. Iron overload (hemosiderosis or concurrent hemochromatosis) sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac, hepatic, or pancreatic insufficiency.
4. Prior bone marrow or stem cell transplant.
5. Clinically symptomatic cholelithiasis or cholecystitis. (Prior cholecystectomy is not exclusionary. Patients with symptomatic cholelithiasis or cholecystitis may be rescreened once the disorder has been treated and clinical symptoms have resolved.)
6. Currently enrolled in another therapeutic clinical trial involving on-going therapy with any investigational or marketed product or placebo. Concurrent participation in the Pyruvate Kinase Deficiency Natural History Study (NCT02053480) is permitted.
7. Exposure to any investigational drug, device, or procedure within 28 days prior to Screening.
8. Concurrent medical condition that could compromise participation in the study such as:
    a. Poorly controlled hypertension (defined as systolic blood pressure (BP) > 150 mm Hg or diastolic BP > 90 mm Hg) refractory to medical management.
    b. History of recent (within < 6 months from Screening date) congestive heart failure; myocardial infarction or unstable angina pectoris; or hemorrhagic, embolic, or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.
    c. Currently active infection requiring the use of parenteral anti-microbial agents or that is ≥ Grade 3 (CTCAEv4.03) within 6 months of first dose.
    d. A pattern or frequency of post-splenectomy sepsis that in the assessment of the Investigator could reasonably be expected to interfere with the ability of the patient to complete the 24 week study participation.

e. Positive test for hepatitis B surface antigen (HBsAg) or hepatitis C virus (HCV) antibody with signs of active Hepatitis B or C virus infection.

f. Positive test for human immunodeficiency virus (HIV) 1 or 2 antibody.

g. Diabetes mellitus judged to be in poor control by the Investigator or requiring > 3 anti-diabetic agents counting insulin; use of insulin *per se* is not exclusionary.

h. History of any primary malignancy with the exception of: curatively treated non-melanomatous skin cancer; curatively treated cervical or breast carcinoma *in situ*; or other primary tumor treated with curative intent and no known active disease present and no treatment administered during the last 3 years.

9. Undergone major surgery within 6 months of first dose.
10. Current or recent history of psychiatric disorder that in the opinion of the Investigator or Medical Monitor could compromise the ability of the patient to cooperate with study visits and procedures.
11. Use of any of the restricted list of products known to strongly inhibit CYP3A4 metabolism (Appendix 15.3, Table 7) within 5 days prior to Day 1 dosing; or to strongly induce CYP3A4 metabolism (Appendix 15.3, Table 8) within 28 days prior to Day 1 dosing; or to strongly inhibit P-gp transporter (Appendix 15.3, Table 9) within 5 days prior to Day 1 dosing; or digoxin within 5 days prior to Day 1 dosing.
12. Serum bilirubin > ULN attributable to factors other than hemolysis and/or Gilbert's syndrome.
13. Male patients with heart-rate corrected QT (Fridericia's correction factor) QTcF interval > 450 msec, or female patients with QTcF interval > 470 msec with the exception of patients with a left bundle branch block (LBBB). Medical Monitor approval needed in patients with a LBBB.
14. Cardiac dysrhythmias judged as clinically significant by the Investigator or requiring therapy with drugs that are primarily substrates of CYP3A4.
15. History of allergy to sulfonamides if characterized by acute hemolytic anemia, anaphylaxis, or rash of erythema multiforme type or Stevens-Johnson syndrome.
16. Any other medical or psychological condition deemed by the Investigator to be likely to interfere with a patient's ability to understand and sign informed consent; cooperate with study visits, tests, and procedures; or otherwise safely and reliably participate in the study.

## 8.4. Patient Identification and Registration

Patients who are candidates for enrollment into the study will be evaluated for eligibility by the Investigator to ensure that the inclusion and exclusion criteria (see Section 8.2 and Section 8.3, respectively) have been satisfied and that the patient is eligible for participation in this clinical study. The site will submit to the Sponsor an Eligibility form for each eligible patient and the Medical Monitor will confirm eligibility for all patients prior to receipt of the first dose of AG-348.

## 8.5. Patient Randomization

Patients who have been confirmed as eligible will be randomized in an equal ratio to a treatment arm (e.g., 1:1 or 1:1:1 depending on which arms are open). A randomization number will be assigned. The site will provide a request for randomization form (including the patient's confirmed genotype) to a central randomization center. The randomization will be stratified by *PKR* mutation in order to maintain balance across the dose arms for the specific mutations expected to be most frequently enrolled. The *PKR* mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H) and all other mutations ("other"). Since this is an open label study, randomization will not be blinded.

Please refer to the study manual for the randomization procedure.

## 8.6. Patient Withdrawal Criteria

Patients have the right to withdraw from the study at any time for any reason. Patients may withdraw or be withdrawn from study-related procedures and treatments under the following conditions:

- Withdrawal of consent;
- Experiences unacceptable toxicity;
- Development of an intercurrent medical condition that precludes further participation in the trial;
- Patient requires use of a prohibited concomitant medication (Section 9.11.2);
- Investigator decision;
- Protocol violation: non-adherence to protocol requirements;
- Pregnancy;
- Lost to follow-up.

Should a patient decide to withdraw, all efforts will be made to complete and report the protocol-defined study observations up to the time of the patient's withdrawal as completely as possible and to determine the reason for withdrawal.

In the event a patient is withdrawn from the study, the Medical Monitor must be informed. If there is a medical reason for withdrawal, the patient will remain under the supervision of the Investigator until satisfactory health is returned.

When a patient withdraws from the study, the primary reason for discontinuation must be recorded in the appropriate section of the electronic case report form (eCRF) and all efforts will be made to complete and report final study observations as thoroughly as possible.

All AEs should be followed until resolution or for a period of 30 days from the last dose of study drug, whichever is shorter. Patients with toxicity suspected to be related to study drug will continue follow-up until the AE resolves, is declared chronic by the Investigator, or the patient is lost to follow-up.

## 8.7. Replacement of Patients

Patients who drop out of the study prior to completing the first 12 weeks of assigned dosing for reasons other than AEs may be replaced at the Sponsor's discretion.

# 9. STUDY TREATMENT

## 9.1. Description of Study Drug

AG-348 sulfate hydrate capsules will be provided as 25 mg or 100 mg (free-base equivalent) of AG-348 sulfate hydrate without excipients in Swedish orange (25 mg) or white opaque (100 mg) gelatin capsules (size 2 capsules for all dose strengths).

All study drugs are for investigational use only and are to be used only within the context of this study. All study drug products will be supplied by the Sponsor. Please see the Investigator's Brochure for further details regarding study drug.

## 9.2. Study Drug Packaging and Labeling

AG-348 sulfate hydrate capsules are packaged in white, high-density polyethylene (HDPE) induction sealed bottles with a child-resistant screw cap.

Packaging and labeling will be prepared to meet all regulatory requirements.

## 9.3. Study Drug Storage

AG-348 sulfate hydrate drug capsules must be stored at room temperature of 15 to 30° C (59 - 86 °F).

All study drug products must be stored in a secure, limited-access location and may be dispensed only by the Investigator or by a member of the staff specifically authorized by the Investigator.

## 9.4. Method of Assigning Patients to Treatment

Up to a maximum of 25 patients will be randomized to any one of the dosing arms in this study. Randomization will be balanced 1:1 or 1:1:1, depending on whether 2 or potentially 3 arms are open, and will be stratified by *PKR* mutation (see Section 8.5). The dose and schedule of AG-348 each patient receives will be dependent upon which dose arm is open for enrollment when the patient qualifies for and is randomized into the study.

## 9.5. Blinding

This is an open-label study; no blinding methods will be used.

## 9.6. Study Drug Preparation and Administration

For the initial two treatment arms, (Arm 1 and Arm 2), AG-348 will be administered orally BID (approximately every 12 hours with a minimum of 10 hours between doses) over a 24 week treatment period. Starting with Day 1, dosing will be continuous; there will be no rest periods. Patients who do not meet any of the treatment withdrawal criteria (see Section 8.5) may continue treatment for the entire 24-week treatment period.

Patients will be dispensed the appropriate number of Sponsor-packaged, labeled bottles to allow for 28 days of dosing until the next scheduled visit.

Patients will be given a dosing diary to be used for each 28-day dosing period. They should record relevant information regarding their study drug in the diary (e.g., confirmation that each daily dose was taken, reasons for missed doses).

Treatment compliance will be assessed based on return of unused drug and the dosing diary (see Section 9.9).

Patients should be instructed to take their daily dose at approximately the same times each day except for dosing on in-clinic visiting days.

Patients who undergo extensive PK/PD sampling (see Appendix 15.1, Table 5) should be instructed from Week 3 on to bring the AM dose with them for in-clinic visits and to ingest the dose following PK/PD blood draws.

Patients receiving limited PK/PD sampling (see Appendix 15.1, Table 6) should be instructed to bring the AM dose with them for all in-clinic visits and to take the AM dose following PK/PD blood draws.

Patients receiving extensive PK/PD sampling on Day 1 and 15 will also have limited PKPD on other visit days. As a general rule, regardless of extensive or limited schedule, patients will bring in the AM dose for all visits and take this dose following PK/PD blood draws.

A minimum of 10 hours between the AM and PM dose will be required on those dosing days. Each dose should be taken with a glass of water and consumed over as short a time as possible. AG-348 may be taken with or without food. Patients should be instructed to swallow capsules whole and to not chew the capsules. For patients who have difficulty swallowing tablet(s), the Medical Monitor should be contacted to discuss administration.

Patients will receive their first dose of AG -348 in the clinic on Day 1 and then may take the remaining doses on an outpatient basis.

## 9.7. Criteria for Dose Escalation, Dose Modification, or Discontinuation of Study Drug

No intra-patient dose escalations will be permitted in this study unless the DRT decides to re-assign patients' doses and schedule in a terminated arm to match the dose and schedule of another arm of the study. In this case, the patients in the terminated arm will remain in their originally assigned arm; i.e., they will not count towards the enrollment quota of the arm whose dose and schedule is being adopted. All dosing modifications, as outlined below, will be implemented following discussions with the Medical Monitor.

### 9.7.1. Dose Modification for Safety

The Investigator will monitor all patients for safety and tolerability. Modification of the patient's dose of AG-348 will be based on AEs and observed changes in Hb levels (see Section 9.7.2).

**Table 1: Dose Modification for Adverse Events Deemed at Least Possibly Related to AG-348**

| Adverse Events(s) | AG-348 Dose Adjustment |
|---|---|
| Grade 1 | None required. |
| Grade 2 | None required; Investigator and Medical Monitor judgment to manage as for Grade 3. |
| Grade 3 | Suspend dosing;<br>If event resolves to Grade 1 or baseline within approximately 14 days of suspension, resume dosing with 1 dose level reduction (see Table 2 below).<br>If event does not resolve to Grade 1 or baseline within approximately 14 days of suspension, permanently discontinue dosing, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor. |
| Grade 4 | Permanently discontinue dosing, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor. |

Dose modifications for Grade 2 and 3 AEs should be maintained long enough for the Investigator to be confident that the reduced dose is being well tolerated before considering a request of the Medical Monitor to permit subsequent re-escalation to the former dose level. Dosing for an individual patient will be discontinued permanently for Grade 3 and Grade 4 AEs that do not resolve to Grade 1 or baseline within approximately 14 days of suspension of dosing, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor. No patients may be re-escalated to their former dose level after a dose modification without discussion with the Medical Monitor.

It should be noted that if the initial dose of 300 mg q12h BID selected for Arm 1 demonstrates an unacceptable safety profile resulting in multiple subjects undergoing dose modifications, the DRT may exercise its option to re-assign these patients' dose and schedule to match the dose and schedule of another study arm (for example, Arm 2 of the study, or to match the dose and schedule of a [potential] Arm 3, if implemented).

### 9.7.2. Dose Modification for Increase in Hemoglobin Level

It is presently unknown to what magnitude, how rapidly, or even whether, AG-348 will result in increased Hb levels in patients with PK Deficiency across the variety of potential *PKR* mutations that may be encountered during this study. Moreover, it is unknown whether any potential increases in Hb experienced by patients in this study may be a safety risk. As a conservative measure, this study incorporates guidance for dose modification based on potential large increases in Hb levels that exceed the midpoint of the typical normal range by gender. The Investigator will monitor all patients for changes in Hb levels and should adjust the dose of AG-348 as outlined in Table 2 per the following guidelines and with Medical Monitor approval.

- Males: If Hb > 15 g/dL and confirmed with a second test, suspend dosing until Hgb ≤ 13.5 g/dL; then resume dosing with a 1 dose level reduction.
- Females: If Hb > 13.5 g/dL and confirmed with a second test, suspend dosing until Hb ≤ 12.5 g/dL; then resume with a 1 dose level reduction.

- The treating Investigator will discuss with the Medical Monitor questions relating to dose modifications on an as needed basis.

**Table 2: Dose Reduction Table (by Dosing Arm)**

| Dose Group | Starting Dose | 1st Dose Reduction | 2nd Dose Reduction |
|---|---|---|---|
| Arm 1 | 300 mg BID | 200 mg BID | 100 mg BID |
| Arm 2 | 50 mg BID | 25 mg BID | TBD[1] |
| Potential Arm 3 | TBD | To approximately 50-66% of initial dose | To approximately 25-33% of initial dose |

[1] Dose to be determined by Medical Monitor.

### 9.7.3. Stopping Criteria

Dosing for an individual patient will be discontinued permanently for Grade 3 AEs that do not resolve to Grade 1 or baseline within approximately 14 days of suspension of dosing, and for Grade 4 AEs, unless the benefits outweigh the risks of resuming treatment and are approved by the Medical Monitor (Section 9.7.1). Other reasons for treatment termination are provided in Section 8.5.

## 9.8. Duration of Patient Participation

The duration of treatment for all patients on this study will be up to 24 weeks. Patients who safely tolerate and demonstrate one or more indicators of clinical activity of AG-348 may be eligible to immediately roll over to a separate safety extension study for continued treatment.

## 9.9. Treatment Compliance

During in-clinic visits, doses of AG-348 will be ingested by the patient under the supervision of clinical facility personnel. For at-home dosing, patients will be given a dosing diary to be used for the duration of the 24-week treatment period. Patients should record relevant information regarding their study drug in the diary (e.g., confirmation that each daily dose was taken, reasons for missed doses) and return the diary at each study visit.

## 9.10. Study Drug Accountability

Accountability for the study drug at the clinical facility is the responsibility of the Investigator. The Investigator will ensure that the study drug is used only in accordance with this protocol. Where allowed, the Investigator may choose to assign drug accountability responsibilities to a pharmacist or other appropriate individual.

The Investigator or delegate will maintain accurate drug accountability records indicating the drug's delivery date to the site, inventory at the site, use by each patient, and return to Agios or its designee (or disposal of the drug, if approved by Agios). These records will adequately document that the patients were provided the doses as specified in the protocol and should reconcile all study drug received from Agios. Accountability records will include dates, quantities, batch/serial numbers, expiration dates (if applicable), and patient numbers. An

unblinded monitor will review drug accountability at the site on a schedule agreed to by the Sponsor.

Study drug must not be used for any purpose other than the present study.

All unused and used study drug will be retained at the site until it is inventoried by the Study Monitor after database lock has occurred. All used, unused or expired study drug will be returned to Agios or its designee or, if authorized, disposed of at the study site per the site's Standard Operating Procedures (SOPs) and documented. All material containing AG-348 will be treated and disposed of as hazardous waste in accordance with governing regulations.

## 9.11. Prior and Concomitant Medications and Treatments

### 9.11.1. Prior Medications and Procedures

All medications administered and procedures conducted within 28 days prior to the first day of study drug administration are to be recorded on the source documentation and included in the eCRF.

### 9.11.2. Prohibited Concomitant Therapy

All concomitant medications and procedures administered from 28 days before administration of study drug through the last Follow-up Visit (Week 28/Day 197) must be recorded in the appropriate section of the source documentation and eCRF along with dosage information, dates of administration, and reason for use.

The following are prohibited at all times during participation in this study:

- Investigational drugs must be discontinued 28 days prior to the first dose of study drug;
- Products known to strongly inhibit CYP3A4 metabolism (listed in Appendix 15.3, Table 7) must be discontinued within 5 days prior to Day 1 dosing;
- Products known to strongly induce CYP3A4 metabolism (listed in Appendix 15.3, Table 8) must be discontinued within 28 days prior to Day 1 dosing;
- Products known to strongly inhibit P-gp transporter (listed in Appendix 15.3, Table 9) must be discontinued within 5 days prior to Day 1 dosing;
- Digoxin must be discontinued within 5 days prior to Day 1 dosing;
- Hematopoietic stimulating agents (erythropoietins, granulocyte colony stimulating factors, thrombopoietins, etc) must be discontinued no less than 28 days prior to the first dose of study drug. [Folic acid 1 mg orally per day is required for all patients. B12 injections are permitted for patients with a prior diagnosis of B12 deficiency syndromes, but must be repleted to stability of the Hb and mean corpuscular volume (MCV) prior to enrollment in the study];
- Anabolic steroids, including testosterone preparations, administered for anemia must be discontinued no less than 28 days prior to the first dose of study drug;

- As the target population for this study consists of transfusion independent patients and transfusion of blood products could confound key endpoints of the study, blood transfusions of any type must be strictly avoided except in cases of compelling medical need. If medical circumstances permit, the Medical Monitor should be contacted for discussion before any transfusions are administered.
- Other drugs that displace unconjugated bilirubin from albumin.

**Potential Drug Interactions**

Strong inhibitors of CYP3A4 (listed in Appendix 15.3, Table 7) are not permitted for use with AG-348. Based on modeling of AG-348 metabolism and tolerability data from the Phase 1 MAD study, moderate inhibitors of CYP3A4 do not appear to pose a risk to patients dosed with AG-348.

In-vivo data from the Phase 1 MAD show AG-348 induces CYP3A4 and its own metabolism, presumably via CYP3A4. The use of strong CYP3A4 inducers in combination with AG-348 is expected to reduce AG-348's efficacy. Therefore, administration of strong CYP3A4 inducers (listed in Appendix 15.3, Table 8) is not permitted with AG-348.

Strong inhibitors of drug transport (listed in Appendix 15.3, Table 9) are not permitted for use with AG-348.

Digoxin is not permitted for use with AG-348.

Induction of CYP3A4 by AG-348 is expected to reduce the efficacy of certain sensitive co-medications. Co-administration of AG-348 with the drugs in Appendix 15.3, Table 10 should be done with caution, as their efficacy may be reduced.

Of note, women in the trial utilizing oral contraception must utilize barrier methods as per the Inclusion Criteria 14 (Section 8.2) while taking AG-348.

The expected patient co-medications deferoxamine, deferasirox, and oral penicillin are not expected to interact with AG-348.

### 9.11.3. Allowed Concomitant Therapy

Medications and treatments other than those specified above are permitted during the study. All intercurrent medical conditions will be treated at the discretion of the Investigator according to acceptable local standards of medical care. Subjects may receive analgesics, antiemetics, anti-infectives (including penicillins), and antipyretics as medically indicated and consistent with the guidance in the previous two sections. Patients may continue iron chelation therapy with deferoxamine and deferasirox. Patients must continue taking 1 mg of folic acid for the duration of the study.

All concomitant medications, including transfusions of blood products, procedures performed during the study, including those used to treat AEs, will be recorded on the eCRF.

### 9.11.4. Potential for Phototoxicity

AG-348 may cause sensitivity to direct and indirect sunlight. Patients should be warned to avoid direct sun exposure. When exposure to sunlight is anticipated for longer than 15 minutes, the

patient should be instructed to apply factor 30 or higher sunscreen to exposed areas and wear protective clothing and sunglasses.

### 9.11.5. Management of Nausea, Vomiting, and Diarrhea

As the primary objective of this study is to assess the safety and tolerability of up to 24 weeks of AG-348, routine use of prophylactic anti-emetic and antidiarrheal or other classes of medications is prohibited. However, if patients experience AEs relating to nausea, vomiting, or diarrhea these may be treated at the Investigator's clinical discretion with recommended medications as follows:

- Nausea/vomiting: allowed agents include standard clinical dosing with palonosetron (Aloxi), dolasetron (Anzemet), or promethazine (Phenergen). Promethazine is a substrate for CYP2B6, and it is presently unknown if the potential for 2B6 induction after AG-348 dosing could be sufficient to reduce the therapeutic effect of promethazine. Not recommended are aprepitant (Emend; CYP3A4 inhibitor), ondansetron (Zofran; CYP3A4 inducer), chlorpromazine (Thorazine; CYP3A4 inducer), prochlorperazine (Compazine; CYP3A4 substrate, QT prolongation has been reported), and granisetron (Kytril; CYP3A4 substrate, QT prolongation has been reported);
- Diarrhea: recommended management includes standard clinical dosing with Kaopectate or other non-absorbable anti-diarrheals, diphenoxylate/atropine (Lomotil), or loperamide (Imodium). Loperamide is the least preferred choice because it is both a substrate and inhibitor for CYP3A4, a substrate for CYP2B6, and a substrate for P-gp.
- For the use of any medications not specifically mentioned above the Investigator may confer with the Sponsor’s Medical Monitor.

### 9.11.6. Other Restrictions and Precautions

Patients should be advised to refrain from altering their normal exercise routine for the first 28 days of treatment with AG-348 as symptoms of anemia could theoretically temporarily worsen before the eventual potential beneficial effect of AG-348 on the Hb level becomes evident. The elevated blood levels of 2,3-DPG commonly seen in patients with PK Deficiency may produce a right shift in the Hb-O2 dissociation curve. The effect of this is to enhance oxygen delivery at the tissue level, thus counteracting to some extent the physiologic consequences of the anemia. AG-348 was shown to produce rapid decreases (within the first 12 hours following a single dose) in 2,3-DPG in two prior clinical trials with healthy adult male and female volunteers. In patients with PKD who have elevated 2,3-DPG levels, it is theoretically possible that decreases in 2,3-DPG that precede correction of the anemia could result in a temporary decrease of oxygen delivery at the tissue level resulting in clinical symptoms (e.g., increased fatigue).

# 10. STUDY ASSESSMENTS

## 10.1. Schedule of Assessments

The Schedules of Assessments for this study are provided in Appendix 15.1.

After obtaining written informed consent, patients will undergo screening evaluations. The Screening visit is to be conducted within approximately 28 days prior to first dose of study treatment.

During the Treatment period, patients will attend visits at baseline (Day 1), weekly through Week 3 (Days 8, 15, and 22), triweekly starting at Week 6 through Week 12 (Weeks 6, 9, and 12) and monthly through Week 24 (Weeks 16, 20, and 24). Patients who safely tolerate and demonstrate one or more indicators of clinical activity of AG-348 through Week 24 may be eligible to immediately enter a separate extension study for continued treatment upon agreement of the treating Investigator and the Medical Monitor. For patients who finish treatment, Study Discharge will occur 4 weeks (Week 28 or earlier) following the last dose of study treatment at the final follow-up assessment.

Whenever more than one assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (e.g., VS first, ECG, blood draw). The timing of these assessments should allow the PK blood draw to occur at the exact nominal time. The order of procedures may be revised with prior discussion between Sponsor and Site.

Minor adjustments to the timing, number of planned safety monitoring procedures (e.g., VS, ECG, blood draw), and PK/PD assessments may be made during the course of the study based on collected data to ensure appropriate safety monitoring and will not require a protocol amendment. These minor changes will require prior approval from the Sponsor's Medical Monitor (or Responsible Medical Officer) as well as appropriate documentation in the study records. The addition of new safety monitoring procedures or other assessments will require a protocol amendment.

## 10.2. Informed Consent and Confirmation of Eligibility

A complete description of the study is to be presented to each potential patient and a signed and dated informed consent is to be obtained before any study specific procedures are performed.

Patient's eligibility will be confirmed at Screening and within 24 hours prior to study treatment dose. If a patient is determined to be ineligible, the patient will be excluded from participation.

## 10.3. Demographic Data, Medical and Medication History

Patient demographic data, including gender, date of birth, age, race, and ethnicity, will be obtained at Screening. Collection of demographic data will be modified by country regulatory requirements, as appropriate.

Medication history, including all relevant prior medical history and current medical conditions, will be obtained at the Screening assessment and on Day -1; all concomitant medications

administered and procedures conducted within 28 days prior to Day 1 should be reported in the source documentation and eCRF. Investigators will be asked to provide information on the patient's history of any medical diagnoses (e.g., iron overload) and surgical procedures (e.g., splenectomy, cholecystectomy) pertaining to their diagnosis of PK deficiency and prior available complete blood counts (CBCs) over the preceding 6 months and transfusion history over the preceding 12 months prior to the date of signing informed consent.

## 10.4. PKR Enzymatic Assay and PKR Genotyping

Assessments for PKR enzymatic activity and PKR genotyping will be performed at Screening only for confirmation of study eligibility. PKR enzymatic assays will be conducted at [redacted]. PKR genotyping will be conducted at [redacted]

## 10.5. Safety Assessments

### 10.5.1. Physical Examination, Height, and Weight

A complete physical examination (genital and rectal examinations will be performed at the discretion of the Investigator) will be obtained at Screening, Baseline, Week 12, and the Follow up Visit (Week 28). For patients rolling over into extension study, the last physical examination will occur at Week 24. Limited focused physical examinations will be performed at all other visits. Any findings will be recorded on the eCRF. Height will be collected at Screening only.

### 10.5.2. Vital Signs

Vital signs, including systolic and diastolic BP, heart rate, respiratory rate, and temperature, will be obtained according to the according to the Schedule of Assessments (Appendix 15.1). Vital sign measurements will be repeated if clinically significant or machine/equipment errors occur. Out of range BP or heart rate measurements will be repeated at the Investigator's discretion. Any confirmed, clinically significant vital sign measurements will be recorded as AEs.

### 10.5.3. Electrocardiogram

A 12-lead ECG will be obtained according to the Schedule of Assessments (Appendix 15.1). The ECGs will be measured using an ECG machine that calculates the heart rate and measures PR, QRS, QT, QTcB (Bazett correction formula), and QTcF (Frederica's correction). Only QTcF (not QTcB) will be used for determination of eligibility.

The 12-lead ECGs should be obtained following 5 minutes of recumbency. The Screening ECG will be performed at least 7 days prior to Day 1 dosing. ECGs will be repeated if clinically significant abnormalities are observed, if artifacts are present, or if machine/equipment errors occur. Any confirmed, clinically significant ECG findings will be recorded as AEs.

### 10.5.4. Safety Laboratory Assessments

#### 10.5.4.1. Hematology, Serum Chemistry, Coagulation Studies, and Urinalysis

Laboratory values obtained prior to Screening and RBC antibodies obtained at Screening will be performed at a local laboratory. On-study clinical laboratory evaluations are to be performed by

a central laboratory. If Investigators believe that it is clinically indicated to obtain safety laboratory results from their own local laboratories on the day of the subject's visit, and before the results are returned from the designated central laboratory, they are free to exercise their discretion to do so. Investigators should be aware that since the Hb level is a secondary endpoint of the study, it behooves them to minimize blood volumes drawn, and samples for the central lab must still be collected, since it will serve as the official lab result for this study.

Clinical laboratory evaluations are to be collected according to the Schedule of Assessments (Appendix 15.1). In addition, all clinically significant laboratory abnormalities noted on testing will be followed by repeat testing and further investigated according to the judgment of the Investigator. Please note that serum free and total testosterone will be collected at 2 time points during Screening at least 2 days apart in addition to Week 1/Day 1 (total of 3 time points prior to Day 1 dosing).

The following safety laboratory parameters are to be determined:

| | |
|---|---|
| **Hematology**: | HCT, Hb, RBC count, absolute reticulocyte count, percent reticulocyte count, MCV, mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count with manual differential, ANC, and absolute lymphocyte count (ALC), and platelet count. G6PD and RBC antibody screen will be performed at Screening only |
| **Other** | EPO, Hp, COHb |
| **Serum Chemistry:** | alkaline phosphatase (ALP), sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide ($CO_2$), albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, LDH, ALT, AST, total bilirubin, and indirect bilirubin. |
| **Sex Hormones**: | testosterone (total and free), estrone, and estradiol. FSH will only be performed at Screening for female patients only for confirmation of post-menopausal status. |
| **Bone Turnover** | serum NTX and CTX. |
| **Lipids** | total cholesterol, HDL-C, triglycerides. |
| **Iron Panel** | iron (Fe), total iron-binding capacity (TIBC), percent saturation, ferritin. |
| **Coagulation Studies:** | fibrinogen, aPTT, INR. |
| **Urinalysis**: | color and appearance, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, pregnancy screen (dipstick), occult blood. Microscopic inspection of sediment will only be performed for cause or to investigate an abnormal dipstick finding per the Investigator’s discretion. |

#### 10.5.4.2. Screening Serology

A blood sample for serology, including HBsAg, HCV Ab screen, and HIV1 and HIV2 Ab, is to be collected from all patients at Screening.

### 10.5.5. Adverse Events

Each patient will be carefully monitored for the development of any AEs throughout the study from signing of the informed consent through all scheduled study follow-up visits, or withdrawal of consent, whichever occurs first. In addition, SAEs that are assessed as possibly or probably related to study treatment that occur > 30 days post-treatment also are to be reported.

AEs will be evaluated by the Investigator and recorded as described in the Schedule of Assessments. On dosing visits, all AEs (elicited and spontaneously reported) will be continuously evaluated by the Investigator and recorded. At any non-dosing day visit, AEs will be evaluated by the Investigator and recorded. Any AEs already documented at a previous assessment and designated as ongoing will be reviewed at subsequent visits or assessment time points as necessary. If these AEs have resolved, this will be documented.

All AEs will be graded using the CTCAE grading system (Appendix 15.2 ).

Complete details on AE monitoring are provided in Section 11.

## 10.6. Pharmacokinetic Assessments

### 10.6.1. Blood Sample Collection and Pharmacokinetic Measurements During Dose Escalation

The first approximately 10 patients treated, contingent on clinical site feasibility, will undergo extensive PK sampling as detailed in Appendix 15.1, Table 5. The remainder of treated patients will undergo limited PK sampling as detailed in Appendix 15.1, Table 6. In-clinic visits on Day 8 and Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis; in these instances PK sampling would not be required.

The collection times for post-dose PK samples will start from the time that dosing is completed. (For example, a PK draw at 30 minutes will be collected 30 minutes after the last capsule has been ingested.) The completion time of each dose will be recorded.

Procedures for sample collection and processing will be provided in a separate study manual. The actual time point of each sample collection will be recorded.

Samples for PK and PD assessments may be retained for up to 2 years from collection.

## 10.7. Pharmacodynamic Assessments

The first approximately 10 patients treated, contingent on clinical site feasibility, will undergo extensive PD sampling for 2,3-DPG and ATP as detailed in Appendix 15.1, Table 5. The remainder of treated patients will undergo limited PD for 2,3-DPG and ATP sampling as detailed in Appendix 15.3, Table 6.

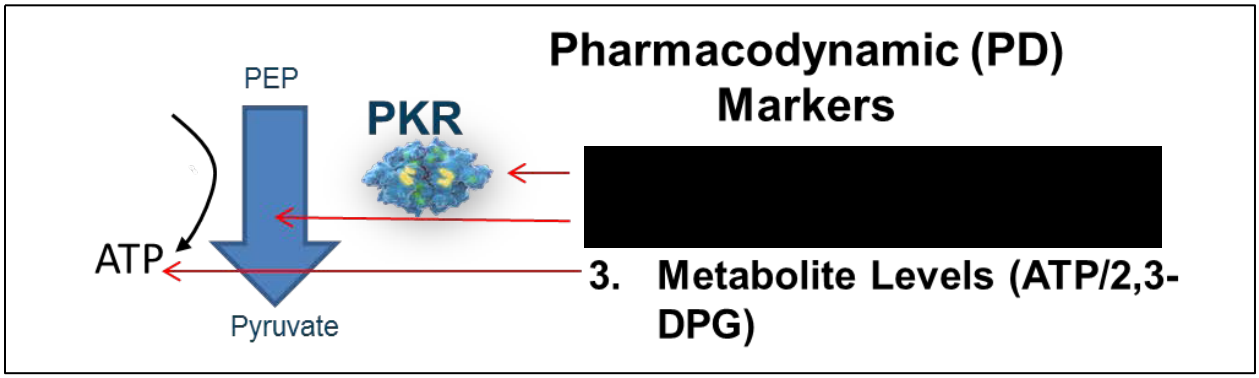
The collection times for post-dose PD samples will start from the time that dosing is completed. (For example, a PD draw at 30 minutes will be collected 30 minutes after the last capsule has been ingested.) The completion time of each dose will be recorded.

Procedures for sample collection and processing will be provided in a separate study manual. The actual time point of each sample collection will be recorded.

Pharmacodynamic assessments will include 2,3-DPG, ATP, [redacted] In-clinic visits on Day 8 and Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis; in these instances PK sampling would not be required.

Figure 4 provides a brief schematic outlining the PKR reaction and how each of these PD assessments fits into a complete mechanistic understanding of the action of AG-348.

**Figure 4: PKR Enzymatic Reaction**




The PKR enzyme catalyzes the PEP to pyruvate reaction, with concomitant formation of ATP.

17. Binding of AG-348 to the PKR tetramer can be assessed through an ex-vivo biochemical assay of cell lysates from AG-348 treated patients. Because WBCs contain a high level of pyruvate kinase from a non-PKR pyruvate kinase isoform, WBCs are first removed by filtration before the purified red cells are frozen. [redacted]
18. [redacted]
19. AG-348 target engagement [redacted] has been shown in preclinical models and healthy volunteer clinical studies to result in accumulation of ATP and depletion of the upstream metabolite 2,3-DPG. Therefore,

levels of these metabolites will be measured by mass spectrometry in frozen whole blood samples.

The first approximately 10 patients treated, contingent on clinical site feasibility, will undergo extensive PD sampling as detailed in Appendix 15.1, Table 5. The remainder of treated patients will undergo limited PD sampling as detailed in Appendix 15.1, Table 6. The collection times for post-dose PD samples will start from the time that dosing is completed. (For example, a PD draw at 30 minutes will be collected 30 minutes after the last capsule has been ingested.) The completion time of each dose will be recorded.

Blood samples will be stored at the site and regularly transported at -80°C ±10 C to the bioanalytical laboratory for analysis. Procedures for sample collection and processing will be provided in a separate study manual.

## 10.8. Ordering of Blood Sample Collection

When more than 1 blood sample is collected at the same nominal time, the samples will be collected in the following order:

1. Safety laboratory assessments
2. PK
3. PD (2,3 DPG, ATP)

[redacted]

## 10.9. Sample Processing, Storage, and Shipment

Instructions for the processing, storage and shipment of all study samples for central analysis will be provided in a separate study manual.

# 11. ADVERSE EVENTS

Monitoring of AEs will be conducted throughout the study. Adverse events and SAEs will be recorded in the source documentation and eCRF from time of the signing informed consent through the Follow-up Visits. All AEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

All SAEs will be followed through 30 days after the last dose of study treatment or until the SAE has resolved. Any SAEs that are assessed as possibly or probably related to study treatment that occur > 30 days post-treatment also are to be reported.

## 11.1. Definition of Adverse Events

### 11.1.1. Adverse Event (AE)

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An AE can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

### 11.1.2. Suspected Adverse Reaction

A suspected adverse reaction is any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of expedited safety reporting, 'reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the AE.

### 11.1.3. Unexpected Adverse Event

An unexpected AE is one for which the nature or severity of the event is not consistent with the applicable product information, e.g., the Investigator's Brochure.

### 11.1.4. Serious Adverse Event

An AE or suspected adverse reaction is considered serious (SAE) if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death;
- Life-threatening. Life-threatening means that the patient was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form;
- In-patient hospitalization or prolongation of existing hospitalization. Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered AEs if the illness or disease existed before the patient was enrolled in the study, provided that it did not deteriorate in an unexpected manner during the study (e.g., surgery performed earlier than planned);

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- Congenital anomaly/birth defect;
- Important medical event. An important medical event is an event that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the patient or patient and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions for SAEs. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in in-patient hospitalization, or the development of drug dependency or drug abuse.

#### 11.1.4.1. Potential Severe Drug-Induced Liver Injury

The document entitled FDA Guidance for Industry, Drug-Induced Liver Injury: Premarketing Clinical Evaluation (FDA 2009) provides guidance on how the measurement of various laboratory parameters may be used to assess a given drug's potential to cause severe liver injury (i.e., irreversible liver failure that is fatal or requires liver transplantation). Such cases are suggested by the following three components:

1. The drug causes hepatocellular injury, generally shown by a higher incidence of 3-fold or greater elevations above the ULN of ALT or AST than the (non-hepatotoxic) control drug or placebo;
2. Among trial patients showing such aminotransferase elevations, often with aminotransferases much greater than 3×ULN, one or more also show elevation of serum total bilirubin to > 2×ULN, without initial findings of cholestasis (elevated serum elevated serum ALP);
3. No other reason can be found to explain the combination of increased aminotransferases and total bilirubin, such as viral hepatitis A, B, or C; pre-existing acute liver disease; or another drug capable of causing the observed injury.

Clinical safety laboratory results compatible with the definition of drug-induced liver injury (DILI) stated above must be repeated for confirmation as soon as possible, and if confirmed, will be scored as an unacceptable AE and reported to FDA as a serious unexpected AE.

## 11.2. Procedures for Reporting Adverse Events and Serious Adverse Events

Each patient must be carefully monitored for the development of any AEs. This information should be obtained in the form of non-leading questions (e.g., "How are you feeling?") and from signs and symptoms detected during each examination, observations of study personnel, and spontaneous reports from patients.

All AEs (serious and non-serious) spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures will be recorded in the source documentation and eCRF. Any clinically relevant deterioration in laboratory assessments or other clinical findings is considered an AE

and must be recorded in the appropriate source documentation and eCRF. Treatment-emergent abnormal clinical laboratory results should generally be reported as AEs if there are accompanying symptoms; if additional diagnostic evaluations or medical (including drug therapy) or surgical interventions are undertaken; if a change in study drug dosing or study drug discontinuation is required; or, if the laboratory result is considered clinically significant by the Investigator. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

All SAEs that occur during the course of the study must be promptly reported by the Investigator to [redacted] Global Safety and Pharmacovigilance (see below). Deaths and AEs assessed as life-threatening are to be reported immediately and SAEs that meet other criteria are to be reported within 24 hours from the point in time when the Investigator becomes aware of the SAE. All SAEs must be reported whether or not they are considered causally related to AG-348. Serious adverse event forms will be completed and the information collected will include subject number, a narrative description of the event, and an assessment by the Investigator as to the severity of the event and relatedness to study drug. Follow-up information on the SAE may be requested by the Sponsor or Medical Monitor.

**Contact Information:**

[redacted] **Safety Management**

SAE Hotline (United States): [redacted]
SAE Fax (United States): [redacted]

SAE Hotline (France): [redacted]
SAE Fax (France): [redacted]

SAE Hotline (Netherlands): [redacted]
SAE Fax (Netherlands): [redacted]

SAE Hotline (Italy): [redacted]
SAE Fax (Italy): [redacted]

SAE Hotline (United Kingdom): [redacted]
SAE Fax (United Kingdom): [redacted]

E-mail: [redacted]

If there are serious, unexpected adverse drug reactions associated with the use of AG-348, the Sponsor will notify the appropriate regulatory agency(ies) and all participating Investigators on an expedited basis. The local Institutional Review Board (IRB)/Independent Ethics Committee (IEC) will be promptly notified based on local regulations where required by the IRB/IEC of all serious, unexpected adverse drug reactions involving risk to human subjects.

All AEs, whether serious or not, will be described in the source documents in the database. All new events, as well as those that worsen in intensity or frequency relative to baseline, which occur after signing the informed consent through the final Follow-up Visit (Day 29 ± 3 days) must be recorded. Adverse events that are ongoing at the time of treatment discontinuation should be followed up to 30 days after the last dose of study treatment. All SAEs will be followed up to 30 days after the last dose of study treatment, or until the SAE has resolved.

Information to be reported in the description of each AE includes:

- A medical diagnosis of the event (if a medical diagnosis cannot be determined, a description of each sign or symptom characterizing the event should be recorded);
- The date of onset of the event;
- The date of resolution of the event;
- Whether the event is serious or not;
- Intensity of the event (see below for definitions);
- Relationship of the event to study treatment (see below for definitions);
- Action taken: none; change in the study drug administration (e.g., temporary interruption in dosing); drug treatment required; non-drug treatment required; hospitalization or prolongation of hospitalization required (complete SAE page); diagnostic procedure performed; patient discontinued from the study (complete End of Study visit);
- Outcome: patient recovered without sequelae; patient recovered with sequelae; event ongoing; patient died (notify the Medical Monitor immediately, and complete the SAE form).

**Intensity** of all AEs will be graded according to the NCI CTCAE Version 4.03 (Appendix 15.1).

**Relationship** to study drug administration will be determined by the Investigator according to the following criteria:

- **Not Related**: Exposure to the study treatment did not occur, or the occurrence of the AE is not reasonably related in time, or the AE is considered unlikely to be related to the study treatment.
- **Possibly Related**: The study treatment and the AE were reasonably related in time, and the AE could be explained equally well by causes other than exposure to the study treatment. An attribution of possibly related means that there are facts in evidence to suggest a possible relationship.
- **Probably Related**: The study treatment and the AE were reasonably related in time, and the AE was more likely explained by exposure to the study treatment than by other causes, or the study treatment was the most likely cause of the AE. An attribution of probably related means that there are facts in evidence to suggest a probable relationship.

For the purpose of safety analyses related to final database review, all AEs that are classified as possible or probable will be considered treatment-related AEs.

## 11.3. Pregnancy Reporting

Pregnancy is neither an AE nor an SAE, unless a complication relating to the pregnancy occurs (e.g., spontaneous abortion, which may qualify as an SAE). However, any pregnancy in a participating female patient or a female partner of a participating male patient that occurs during this study or within 30 days following the last dose of AG-348 must be reported to the Sponsor

or Medical Monitor within 30 days of being notified of the pregnancy. The Investigator must follow up and document the course and outcome of all pregnancies even if the patient was discontinued from the study or if the study has finished. The female patient or partner of a male patient should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus. Monitoring should continue until conclusion of the pregnancy.

All outcomes of pregnancy must be reported by the Investigator to the Sponsor or Sponsor's designee on a Pregnancy Outcome Report form within 30 days after he/she has gained knowledge of the delivery or elective abortion.

Any SAE that occurs during pregnancy must be recorded on the SAE report form (e.g., maternal serious complications, spontaneous or therapeutic abortion, ectopic pregnancy, stillbirth, neonatal death, congenital anomaly, or birth defect) and reported within 24 hours in accordance with the procedure for reporting SAEs.

All patients, male and female, must agree to use effective contraception during the entire study and for 30 days following the last dose of AG-348.

# 12. STATISTICAL METHODS

The primary objective of this Phase 2 study is to evaluate the safety and tolerability of up to 24 weeks of AG-348 in patients with PK Deficiency. Therefore, analyses will be primarily descriptive in nature; no formal hypothesis testing will be conducted. All analyses will be conducted separately within each dose arm, or pooled where appropriate.

## 12.1. Sample Size Estimation

Due to the rare disease setting, the minimal sample size in each dose arm may be determined by feasibility. In order to evaluate the primary objective of safety and tolerability of AG-348 in Arm 1 and Arm 2, up to a maximum of 25 patients may be randomized onto each arm. The actual number of patients enrolled into Arms 1 and 2 will depend on the safety reviews and decisions made by the DRT. In addition, up to 25 additional patients may be enrolled to evaluate an additional dose arm (Arm 3; see Section 7.1). As for Arms 1 and 2, the actual enrollment in a potential Arm 3 will depend on the safety reviews and decisions made by the DRT. Therefore, up to approximately 75 total patients may be enrolled in this study across 2 to 3 dose arms.

Table 3 provides the probability within a dose arm of detecting 1 or more AEs with varying sample size and the true underlying AE rates.

**Table 3: Sample Size Estimation**

| Sample Size | True Underlying AE Rate | | |
|---|---|---|---|
| | **15%** | **10%** | **5%** |
| 5 | 56% | 41% | 23% |
| 10 | 80% | 65% | 40% |
| 15 | 91% | 79% | 54% |
| 25 | 98% | 93% | 72% |

## 12.2. Populations for Analysis

The following patient populations (i.e., analysis sets) will be evaluated and used for presentation of the data:

- Safety Analysis Set: All patients who are enrolled and receive any dose of study treatment. The Safety Analysis Set will be the primary set for the analysis of safety data. Patients will be classified according to treatment received, where treatment received is defined as the assigned treatment if it is received at least once, or as the first treatment received if assigned treatment is never received. Unless otherwise stated, the Safety Analysis Set will be the default analysis set for all data analyses.
- Pharmacokinetic (PK) Analysis Set: All patients in the Safety Analysis Set with sufficient plasma sample data to assess PK parameters. Results of the potential PD activity of AG-348 will also be based on the PK analysis set.

- Efficacy Analysis Set: All patients who are enrolled and achieve at least 50% compliance with their assigned dose intensity for at least 4 weeks of continuous dosing. Additional efficacy analyses may be performed on subsets of patients with other degrees of compliance with their assigned dose intensity and/or duration of dosing. The Efficacy Analysis Set will be the primary set for the analysis of preliminary clinical activity data. Patients will be classified according to assigned treatment.

[redacted] If such analyses are performed, they will be described in a separate PK Statistical Analysis Plan (SAP) and may be reported separately in a stand-alone report.

## 12.3. Procedures for Handling Missing, Unused, and Spurious Data

No imputation will be performed for missing data elements. When summarizing AE data, partial dates will be imputed as described in the SAP. Additional rules addressing the handling of missing data will be detailed in the SAP.

## 12.4. Interim Analysis

No formal statistical interim analysis will be conducted. Safety data will be reviewed on an ongoing basis by the DRT, who will meet to review safety, PK, PD, and preliminary clinical activity data at regular intervals (approximately every 6 weeks) throughout the duration of the study. The DRT's decisions to suspend, terminate, or open a potential third dosing arm, or re-assign patients' dosing in a terminated arm to match the dose and schedule of another arm of the study will be based on the totality of the data including, safety, PK, PD, and preliminary clinical activity (e.g., changes in Hb levels).

Additional interim reviews of data may be conducted to support decision making concerning the current clinical study, the sponsor's development programs in general, or in case of any safety concerns.

## 12.5. Statistical Methodology

### 12.5.1. General Methods

This study will be primarily descriptive in nature; therefore, there will be no formal hypothesis testing. Summaries will be produced for disposition, baseline disease characteristics and demographic data including genotype, safety measurements, PK, PD parameters and indicators of clinical activity. Data from each AG-348 dose group will be analyzed separately, and pooled across all dose groups where appropriate. All data will also be listed by individual patient.

Categorical variables will be summarized by frequency distributions (number and percentages) and continuous variables will be summarized by descriptive statistics (mean, standard deviation [SD], median, minimum, and maximum).

### 12.5.2. Disposition

A summary of the disposition of patients will be presented, including the number enrolled, the number treated, and the reasons for study discontinuation. Entry criteria and protocol deviations will be listed.

### 12.5.3. Exposure and Safety Analyses

Patients will receive multiple PO doses of AG-348 over a 24-week treatment period. The actual dose and duration in days of AG-348, as well as the dose intensity (computed as the ratio of actual dose received and actual duration) and the relative dose intensity (computed as the ratio of dose intensity and planned dose received/planned duration) will be listed and summarized using descriptive statistics by dose arm.

Adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and the incidence of TEAEs (new or worsening from baseline) will be summarized by primary system organ class (SOC), preferred term (PT), CTCAE Version 4.03 severity, outcome, action taken with study drug, and relationship to the study drug by dose group. Separate summaries will be produced for all TEAEs, treatment-related AEs (those considered by the Investigator as at least possibly drug related), SAEs, discontinuations due to AEs, and AEs ≥ Grade 3 severity. Individual patient listings will be provided for deaths, SAEs, AEs leading to interruption and/or reduction of study drug dose, and AEs leading to discontinuation of treatment.

Analyses of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined as any AE with onset beginning at the day of first administration of study drug, throughout the treatment period until 30 days after the last dose of study drug, or any event that was present at baseline but worsened in intensity or was subsequently considered drug-related by the Investigator through the end of the study.

Descriptive statistics will be provided for clinical laboratory values (e.g., hematology, serum chemistry, coagulation studies, urinalysis) and VS data, presented as both actual values and changes from baseline relative to each on-study evaluation. Shift analyses will be conducted for selected laboratory parameters based on the baseline CTCAE grade to maximum CTCAE grade. Where applicable CTCAE terms do not exist, a grading system based on the upper and/or lower limits of normal will be used to classify the results.

No formal hypothesis-testing analysis of AE incidence rates will be performed.

Electrocardiogram analyses will include individual patient listings and summaries of abnormal and clinically significant ECG results. Actual values and changes from baseline in PR, QRS, and heart-rate corrected QT interval (QTc) intervals will be summarized by visit and dose arm. Full details of the QTc analysis including correction methods used will be described in the SAP.

Concomitant medications will be coded using the WHO Drug Dictionary. Concomitant medications prior to and after the start of the study drug will be listed by patient, and summarized by ATC term and dose arm.

### 12.5.4. Pharmacokinetic Analyses

Descriptive statistics will be used to summarize PK parameters for the parent compound AG-348 and the metabolite AGI-8702 for each dose group, and where appropriate, for the entire

population. Pharmacokinetic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum and maximum, geometric mean, and geometric coefficient of variation. PK analyses will be described in a separate PK SAP.

### 12.5.5. Pharmacodynamic Analyses

Descriptive statistics will be used to summarize PD parameters for 2,3-DPG and ATP for each dose group, and where appropriate for the entire population. Pharmacodynamic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation %, median, minimum, and maximum, geometric mean, and geometric coefficient of variation %. PD analyses will be described in a separate PD SAP.

### 12.5.6. Aromatase Hormone Analysis

The analyses of serum sex hormones will use appropriate graphic displays and statistical analyses to evaluate patient change from baseline for each parameter. These analyses will include summaries of actual values and change from baseline using appropriate descriptive statistics (mean, SD, median, min and max) as described above.

[redacted] These analyses will present information by each dose arm, and analyses of a pooled AG-348 cohort. Additional details regarding these analyses will be provided in the SAP.

### 12.5.7. Clinical Activity

Details on analyses to evaluate indicators of potential clinical activity of AG-348 in patients with PK Deficiency will be described in the SAP. These will include changes in Hb, HCT, reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, ferritin, and transferrin saturation (serum iron/iron binding capacity). Characterization of Hb response which will include, but is not limited to percent of patients with increase in Hb, time to Hb response, and duration of Hb response will be explored.

[redacted]

## 12.6. Procedures for Reporting Deviations to Original Statistical Analysis Plan

All deviations from the original SAP will be provided in the final clinical study report.

# 13. ADMINSTRATIVE REQUIREMENTS

## 13.1. Good Clinical Practice

The study will be conducted in accordance with the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s). The Investigator will be thoroughly familiar with the appropriate use of the study drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study, and retained according to the appropriate regulations.

## 13.2. Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki (Appendix 15.4).

The Investigator must obtain IRB approval for the investigation and must submit written documentation of the approval to the Sponsor before he or she can enroll any patient into the study. The IRB will review all appropriate study documentation in order to safeguard the rights, safety, and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, Investigator's Brochure, informed consent, advertisements (if applicable), written information given to the patients, signed Dose Escalation Interim Safety Reports, annual progress reports, and any revisions to these documents will be provided to the IRB. The IRB is to be notified of any amendment to the protocol in accordance with local requirements. Progress reports and notifications of serious unexpected adverse drug reactions are to be provided to the IRB according to local regulations and guidelines.

## 13.3. Patient Information and Informed Consent

The Investigator or trained designee will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk, and benefit of the study. Patients must also be notified that they are free to discontinue from the study at any time. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

After the study has been fully explained, written informed consent will be obtained from the patient prior to study participation.

The patient's signed and dated informed consent must be obtained before conducting any study-related procedures. The Investigator must maintain the original, signed consent form. A copy of the signed form must be given to the patient.

The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

## 13.4. Patient Confidentiality

In order to maintain patient privacy, all source documents, study drug accountability records, study reports and communications will identify the patient by the assigned patient number. The Investigator will grant monitor(s) and auditor(s) from the Sponsor or its designee and regulatory authority(ies) access to the patient's original medical records for verification of data gathered on the source documents and to audit the data collection process. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

## 13.5. Protocol Compliance

The Investigator will conduct the study in compliance with the protocol. Modifications to the protocol should not be made without agreement of both the Investigator and the Sponsor. Changes to the protocol will require written IRB approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to patients. The IRB may provide, if applicable, where regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval/favorable opinion of the IRB. The Sponsor or designee will submit all protocol modifications to the regulatory authority(ies) in accordance with the governing regulations.

When immediate deviation from the protocol is required to eliminate an immediate hazard(s) to patients, the Investigator will contact the Sponsor's Medical Monitor (or Responsible Medical Officer), if circumstances permit, to discuss the planned course of action. Any departures from the protocol must be fully documented in the source documents/database.

## 13.6. Data Management

A paper or eCRF will be completed for each patient or an electronic data capture system will be used. The electronic data capture system (EDC) (ClinBase™) is a software tool designed to ensure quality assurance and facilitate data capture during clinical trials. Through a system regulated workflow that includes barcode scanning and interfaces to medical equipment to avoid manual data entry, study operations performance is controlled and captured in real time. The system is fully Code of Federal Regulations (CFR) 21 Part 11 compliant. Source documentation supporting the data should indicate participation in the study and should document the dates and details of study procedures, AEs, and patient status. The Investigator, or trained designee should complete and the Investigator should verify the source documents as the information is collected. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data. The Investigator will ensure the accuracy, completeness, and timeliness of the data reported to the Sponsor. Data collection processes and procedures will be reviewed and validated to ensure completeness, accuracy, reliability, and consistency. A complete audit trail will be maintained of all data changes. The Investigator or designee will cooperate with the Sponsor's representative(s) for the periodic review of study documents to ensure the accuracy and completeness of the data capture system at each scheduled monitoring visit. Electronic consistency checks and manual review will be used to identify any errors or inconsistencies in the data. This information will be provided to the respective study sites by means of electronic or manual queries. The Investigator or designee will prepare and maintain adequate and accurate study documents (medical records, ECGs, AE, and concomitant

medication reporting, raw data collection forms, etc.) designed to record all observations and other pertinent data for each patient receiving study treatment. The Investigator will allow Sponsor representatives, contract designees, authorized regulatory authority inspectors, and the IRB to have direct access to all documents pertaining to the study.

## 13.7. Source Document/Case Report Form Completion

Source documents will be completed for each study patient. It is the Investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the patient's source document. The source document should indicate the patient's participation in the study and should document the dates and details of study procedures, AEs and patient status.

The Investigator, or designated representative, should complete the source document as soon as possible after information is collected, preferably on the same day that a study patient is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.

The Investigator will sign and date each required assessment for all study patients. The Investigator will retain all completed source documents.

## 13.8. Direct Access to Source Data

The study will be monitored by the Sponsor or its designee. Monitoring will be done by personal visits from a representative of the Sponsor (site monitor) and will include on-site review of the source documents for completeness and clarity, cross-checking with source documents, and clarification of administrative matters will be performed. The review of medical records will be performed in a manner to ensure that patient confidentiality is maintained.

The site monitor will ensure that the investigation is conducted according to protocol design and regulatory requirements by frequent communications (letter, telephone, e-mail, and fax).

All unused study drug and other study materials are to be returned to the Sponsor or designee after the study has been completed and the database has been locked.

Regulatory authorities, the IRB, and/or the Sponsor's clinical quality assurance group or designee may request access to all source documents, database, and any other applicable study documentation for an on-site audit or inspection. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities.

## 13.9. Record Retention

The Investigator will maintain all study records according to ICH-GCP and applicable regulatory requirement(s). Records will be retained for at least 2 years after the last marketing application approval or 2 years after formal discontinuation of the clinical development of the investigational product or according to applicable regulatory requirement(s). If the Investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility. The Sponsor must be notified in writing if a custodial change occurs.

## 13.10. Liability and Insurance

The Sponsor has subscribed to an insurance policy covering, in its terms and provisions, its legal liability for injuries caused to participating persons and arising out of this research performed strictly in accordance with the scientific protocol as well as with applicable law and professional standards.

## 13.11. Publication of Study Findings and Use of Information

All information regarding AG-348 supplied by the Sponsor or designee to the Investigator is privileged and confidential information. The Investigator agrees to use this information to accomplish the study and will not use it for other purposes without consent from the Sponsor. It is understood that there is an obligation to provide the Sponsor with complete data obtained during the study. The information obtained from the clinical study will be used towards the development of AG-348 and may be disclosed to regulatory authority(ies), other Investigators, corporate partners, or consultants as required.

# 14. LIST OF REFERENCES

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van Wijk R, and van Solinge WW. The energy-less red blood cell is lost: erythrocyte enzyme abnormalities of glycolysis. *Blood.* 2005;106(13):4034-42.

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# 15. APPENDICES

## 15.1. Schedules of Assessments

### Table 4: Schedule of Assessments:

| Timing | Pre-Treatment | Month 1 | | | | Months 2 and 3 | | | Months 4, 5 and 6 | | | Follow Up |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | **Baseline D1** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day** | **-28 to -1** | **1** | **8[1]** | **15** | **22[1]** | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window** | | | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| Written Informed Consent | X | | | | | | | | | | | |
| PK enzyme assay (confirmation of PK Deficiency) | X | | | | | | | | | | | |
| PKR Genotype (for randomization) | X | | | | | | | | | | | |
| UGT1A1 Genotype | X | | | | | | | | | | | |
| Demographics | X | | | | | | | | | | | |
| Medical/Surgical History (General and PK Deficiency-specific)[2] | X | | | | | | | | | | | |
| Medication History | X | | | | | | | | | | | |
| Transfusion History | X | | | | | | | | | | | |
| Confirmation of Vaccinations (Splenectomized Patients) | X | | | | | | | | | | | |
| Physical Examination[3]/ Height[3] and Weight | X | X | | | X | | X | X | X | X | X | X |
| Performance Status | X | X | | | X | | X | X | X | X | X | X |
| Vital signs (BP, HR, RR, T)[4] | X | X | X | X | X | X | X | X | X | X | X | X |
| 12-lead ECG[5] | X | X | X | | X | | | | | | X | X |
| Laboratory Evaluations[6]: | | | | | | | | | | | | |
| HBsAg, HCV Ab, HIV1 and 2 Ab | X | | | | | | | | | | | |
| RBC antibody Screen | X | | | | | | | | | | | |

| Timing | Pre-Treatment | Month 1 | | | | Months 2 and 3 | | | Months 4, 5 and 6 | | | Follow Up |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | **Baseline D1** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day** | **-28 to -1** | **1** | **8[1]** | **15** | **22[1]** | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window** | | | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| Hematology (CBC)[7] | X | X | X | X | X | X | X | X | X | X | X | X |
| Haptoglobin[8] | | X | | | X | | | X | | | X | X |
| EPO levels[9] | | X | | | X | | | X | | | X | X |
| G6PD screen | X | | | | | | | | | | | |
| Serum Chemistry[10] | X | X | X | X | X | X | X | X | X | X | X | X |
| Iron Panel[11] | | X | | | | | | X | | | X | |
| Carboxyhemoglobin (COHb) | | X | | | X | X | X | X | X | X | X | |
| Coagulation Studies[12] | X | X | | | X | | | X | | | X | X |
| Urinalysis[13] | X | X | | | X | | | X | | | X | X |
| Serum or Urine Pregnancy[14] | X | X | | | | | | | | | | |
| Lipids[15] | | X | | | | X | | X | | | X | X |
| Hormonal Testing[16] | X | X | | | | | | X | | | X | X |
| Serum NTX and CTX | | X | | | | | | X | | | X | |
| Randomization[17] | X | | | | | | | | | | | |
| Study Drug Administration | | X | X | X | X | X | X | X | X | X | X | |
| Dispense Study Drug[18] | | X | X | X | X | X | X | X | X | X | | |
| PK blood sampling[19] | | X | | X | X | X | X | X | X | X | X | |
| PD Assessments[19] | | | | | | | | | | | | |
| 2,3 DPG/ATP | | X | | X | X | X | X | X | X | X | X | |
| [redacted] | | | | | | | | | | | | |
| Adverse Events[21] | | Continuous | | | | | | | | | | X |
| Transfusion Record | X | X | X | X | X | X | X | X | X | X | X | X |
| Concomitant Medications | X | X | X | X | X | X | X | X | X | X | X | X |

| Timing | Pre-Treatment | Month 1 | | | | Months 2 and 3 | | | Months 4, 5 and 6 | | | Follow Up |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Screening** | **Baseline D1** | **W1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** | **W28** |
| **Study Day** | **-28 to -1** | **1** | **8[1]** | **15** | **22[1]** | **43** | **64** | **85** | **113** | **141** | **169** | **197** |
| **Visit Window** | | | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| Rollover to extension study | | | | | | | | | | | X | |

Abbreviations: Ab = antibody; ATP = adenosine triphosphate; BP = blood pressure; CBC= complete blood count; COHb = carboxyhemoglobin; CTX = C-terminal telopeptide; D = day; DPG = diphosphoglycerate; ECG = electrocardiogram; EPO = erythropoietin; FSH = follicle stimulating hormone; G6PD = glucose-6-phosphate-dehydrogenase; HBsAg = hepatitis B surface antigen; HCV Ab = hepatitis C virus antibody; HDL-C = high-density lipoprotein-C; HIV = human immunodeficiency virus; HR = heart rate; NTX = N-terminal telopeptide; PD = pharmacodynamic; PK = pharmacokinetic; PKD = pyruvate kinase deficiency; PKR = pyruvate kinase isoform R; RR = resting rate; W = week.

*Whenever more than one assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (e.g., VS, ECG, blood draw). The timing of these assessments should allow the blood draw to occur at the exact nominal time. The order of procedures may be revised with prior discussion between Sponsor and Site.*

[1] In-clinic visits on Day 8 and Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis; in these instances PK/PD sampling would not be required and dispensing of study medication would not be performed.

[2] Medical history, including all relevant prior medical history, current medical conditions, and hematology profile (CBCs) over prior 6 months, will be obtained at the Screening assessment.

[3] A complete physical examinations (genital and rectal examinations will be performed at the discretion of the Investigator) will be obtained at Screening, Baseline, Week 12, and the Follow up Visit (Week 28), or Week 24 for patients rolling over into extension study. Limited focused physical examinations will be performed at all other specified visits. Height to be collected at Screening only.

[4] Vital signs will include systolic and diastolic blood pressure, heart rate, respiratory rate, and temperature.

[5] 12-lead ECGs are to be conducted after 5 minutes of recumbency. Screening ECG will be performed at least 7 days prior to Day 1 dosing.

[6] Laboratory evaluations (hematology, serum chemistry, coagulation studies, and urinalysis) are to be collected in the morning. These should be collected following an overnight fast on Baseline Day 1 Week 6 (Day 43), Week 12 (Day 85), Week 24 (Day 169), and Follow-up Week 28 (Day 197), when the lipid samples are also included.

[7] Complete blood count (CBC) to be performed at Screening and prior to dosing on Day 1 and will include HCT, Hb, red blood cell (RBC) count, absolute reticulocyte count, percent reticulocyte count, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count with manual differential, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and platelet count.

[8] Haptoglobin will be performed prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

[9] Erythropoietin (EPO) levels will be performed prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

[10] Alkaline phosphatase, sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide ($CO_2$), albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and indirect bilirubin.

[11] Iron (Fe), total iron-binding capacity (TIBC), percent saturation, and ferritin will be performed prior to dosing on Day 1, at the end of Week 12 and at the end of Week 24.

[12] Fibrinogen, activated partial thromboplastin time (aPTT), and international normalized ratio (INR) will be performed at Screening, prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

[13] Color, appearance, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, and occult blood. Microscopic inspection of sediment should only be performed for cause or to investigate an abnormal dipstick finding per the Investigator's discretion. Urinalysis will be performed at Screening, prior to dosing on Day 1, at the end of Week 3, the end of Week 12, the end of Week 24, and the end of Week 28.

[14] Must be repeated at any point throughout the study period if pregnancy is clinically suspected.
[15] Total cholesterol, HDL-C, and triglyceride samples will be collected in the morning following an overnight fast.
[16] Estrone, estradiol, and testosterone (free and total). The first serum estrone and estradiol samples will be collected on Baseline/Day 1. Serum free and total testosterone will be collected at 2 time points during Screening at least 2 days apart in addition to Baseline/Day 1 (total of 3 time points pre dose on Day 1). Serum estrone, estradiol, and free and total testosterone will then follow the schedule indicated on Weeks 12, 24, and 28. FSH will only be performed at Screening for female patients only for confirmation of post-menopausal status.
[17] Randomization will be performed following *PKR* genotyping and prior to dosing on Day 1.
[18] Study drug will be dispensed on a 28-day schedule, or on an alternate schedule (< 28 days) as needed to accommodate patient visit schedule and dose modifications.
[19] For the first 10 patients treated, extensive PK/PD sampling will be conducted on Days 1 and 15 (see Appendix 15.1, Table 5 for details), followed by limited PK/PD sampling from Week 3 to Week 24 (see Appendix 15.1, Table 6 for details). Limited PK/PD sampling will be conducted on the remainder of patients treated (see Appendix 15.1 , Table 6). See Section 10.6.1, Section 10.7, and Section 10.9 for details on blood sampling for PK and PD assessments, respectively, and guidelines on sample processing and storage.
[redacted]
All patients will be evaluated for AEs from the time they sign informed consent until they complete all scheduled study follow-up visits or withdraw consent, whichever occurs first.

**Table 5: Schedule of Assessments: Extensive PK/PD Sampling**

| Sample Timing/Interval | Month 1 | | | | | | | | Months 2 and 3 | | | Months 4, 5 and 6 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Visit | Baseline / D1<br>W2 / D15 | | | | | | | W3 | W6 | W9 | W12 | W16 | W20 | W24 |
| Study Day | 1/15 | | | | | | | 22 | 43 | 64 | 85 | 113 | 141 | 169 |
| Visit Window | ± 2 D (D15) | | | | | | | ± 2 D | ± 7 D | ± 7 D | ± 7 D | ± 7 D | ± 7 D | ± 7 D |
| Timing | Pre-dose[1] | 30 min[2] | 1 hr[2] | 2 hr[2] | 4 hr[3] | 8 hr[3] | 12 hr[3] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] |
| PK blood sample | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| 2,3 DPG/ATP | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| [redacted] | | | | | | | | | | | | | | |

Abbreviations: ATP = adenosine triphosphate; D = day; DPG = diphosphoglycerate; PD = pharmacodynamic; PK = pharmacokinetic; PKR = pyruvate kinase isoform R; W = week.

[1] The acceptable time window will be within 60 minutes prior to study treatment dose administration for the pre-dose PK/PD sample.
[2] The acceptable time window will be within ± 5 minutes of the scheduled collection time for the 30 minute, 1 and 2 hour PK/PD samples.
[3] The acceptable time window will be within ± 30 minutes of the scheduled collection time for the 4, 8, and 12 hour PK/PD samples.
[4] To be collected on Day 1 only.
[redacted]
[6] If the 12 hour time point cannot be collected at site, an 8 hour time point may be collected instead.

**Table 6: Schedule of Assessments: Limited PK/PD Sampling**

| Sample Timing/Interval | Month 1 | | | Months 2 and 3 | | | Months 4, 5 and 6 | | |
|---|---|---|---|---|---|---|---|---|---|
| **Visit** | **Baseline / D1** | **W2** | **W3** | **W6** | **W9** | **W12** | **W16** | **W20** | **W24** |
| **Study Day** | **1** | **15** | **22** | **43** | **64** | **85** | **113** | **141** | **169** |
| **Visit Window** | **-** | **± 2 D** | **± 2 D** | **± 2 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** | **± 7 D** |
| **Timing** | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] | Pre-dose[1] |
| PK blood sample | X | X | X | X | X | X | X | X | X |
| 2,3 DPG/ATP | X | X | X | X | X | X | X | X | X |
| [redacted] | | | | | | | | | |

Abbreviations: ATP = adenosine triphosphate; D = day; DPG = diphosphoglycerate; PD = pharmacodynamic; PK = pharmacokinetic; [redacted]
W = week.

[1] The predose blood sample for plasma PK/PD analysis should be collected within 60 minutes prior to study treatment dose administration.

## 15.2. National Cancer Institute Common Terminology Criteria for Adverse Events

The NCI CTCAE, Version 4.03, can be accessed using the following link:

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

## 15.3. Potential Drug Interactions

Strong inhibitors of CYP3A4 (listed in Table 7) are not permitted for use with AG-348. Based on modeling of AG-348 metabolism and tolerability data from the Phase 1 MAD study, it is thought that moderate inhibitors of CYP3A4 do not pose a risk to patients dosed with AG-348.

In-vivo data from the Phase 1 MAD show AG-348 induces CYP3A4 and induces its own metabolism, presumably via CYP3A4. The use of strong CYP3A4 inducers in combination with AG-348 is expected to reduce AG-348's efficacy. Therefore, administration of strong CYP3A4 inducers (listed in Table 8) with AG-348 is not permitted. Strong inhibitors of drug transport (listed in Table 9) are not permitted for use with AG-348.

Digoxin is not permitted for use with AG-348.

Induction of CYP3A4 by AG-348 is expected to reduce the efficacy of certain sensitive co-medications. Co-administration of AG-348 with the drugs in Table 10 should be done with caution, as their efficacy may be reduced.

Of note, in accordance with Inclusion Criteria 14, women in the trial utilizing oral contraception must utilize barrier methods while taking AG-348.

The expected patient co-medications deferoxamine, deferasirox and oral penicillin are not expected to interact with AG-348.

**Table 7: Strong and Moderate CYP3A4 Inhibitors**

| Strong CYP3A4 Inhibitors: Contraindicated | Moderate CYP3A4 Inhibitors: No Action |
|---|---|
| Indinavir | Aprepitant |
| Nelfinavir | Erythromycin |
| Ritonavir | Fluconazole |
| Clarithromycin | Grapefruit juice[1] |
| Itraconazole | Verapamil |
| Ketoconazole | Diltiazem |
| Nefazodone | |
| Saquinavir | |
| Suboxone | |
| Telithromycin | |

Strong Inhibitor; > 5 fold increase in AUC
Moderate Inhibitor; > 2 fold, < 5 fold increase in AUC
[1] Although classified as a moderate CYP3A4 inhibitor, grapefruit and grapefruit juice are prohibited.

**Table 8: Strong CYP3A4 Inducers**

| **Strong CYP3A4 Inducers: Contraindicated** | |
|---|---|
| Efavirenz | Phenytoin |
| Nevirapine | Pioglitazone |
| Carbamazepine | Rifabutin |
| Glucocorticoids | Rifampin |
| Modafinil | St. John's Wort |
| Oxcarbazepine | Troglitazone |
| Phenobarbital | |

**Table 9: Strong P-glycoprotein Inhibitors**

| **Strong P-gp Inhibitors: Contraindicated** | |
|---|---|
| Amiodarone | Felodipine |
| Azithromycin | Itraconazole |
| Captopril | Ketoconazole |
| Carvedilol | Lopinavir |
| Clarithromycin | Ritonavir |
| Conivaptan | Quercetin |
| Cyclosporine | Quinidine |
| Diltiazem | Ranolazine |
| Dronedarone | Ticagrelor |
| Erythromycin | Verapamil |

**Table 10: Sensitive CYP3A4 Substrates**

| **Sensitive CYP3A4 Substrates: Substitute or Use with Caution** | | | | |
|---|---|---|---|---|
| **Macrolide antibiotics:** | **Prokinetic:** | **Steroid 6beta-OH:** | | |
| Clarithromycin | Cisapride | Estradiol | Finasteride | Terfenadine |
| Erythromycin | | hydrocortisone | Gleevec | Torisel |
| Telithromycin | **Antihistamines:** | progesterone | Haloperidol | Trazodone |
| | Astemizole | Testosterone | Irinotecan | Vemurafenib |
| **Anti-arrhythmics:** | Chlorpheniramine | | LAAM | Vincristine |
| Quinidine→3-OH | Terfenadine | **Miscellaneous:** | Lidocaine | Zaleplon |
| | | Alfentanil | Methadone | Ziprasidone |
| **Benzodiazepines:** | **Calcium Channel Blockers:** | Aprepitant | Nateglinide | Zolpidem |
| Alprazolam | Amlodipine | Aripiprazole | Nevirapine | |
| Diazepam→3OH | Diltiazem | Boceprevir | Ondansetron | |
| Midazolam | Felodipine | Buspirone | Pimozide | |
| Triazolam | Lercanidipine | Carbamazepine | Propranolol | |
| | Nifedipine | Cafergot | Quetiapine | |
| **Immune Modulators:** | Nisoldipine | Caffeine→TMU | Quinine | |
| Cyclosporine | Nitrendipine | Cilostazol | Risperidone | |
| Tacrolimus (FK506) | Verapamil | Cocaine | Romidepsin | |
| | | Codeine-N-demethylation | Salmeterol | |
| **HIV Antivirals:** | **HMG CoA Reductase Inhibitors**: | Dapsone | Sildenafil | |
| Indinavir | Atorvastatin | Dexamethasone | Sirolimus | |
| Nelfinavir | Cerivastatin | Dextromethorphan | Sorafenib | |
| Ritonavir | Lovastatin | Docetaxel | Sunitinib | |
| Saquinavir | Simvastatin | Domperidone | Tamoxifen | |
| | | Eplerenone | Taxol | |
| | | Fentanyl | Telaprevir | |

## 15.4. Declaration of Helsinki

**WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI:**

Recommendations Guiding Medical Doctors in Biomedical Research Involving Human Subjects

Adopted by the 18th World Medical Association (WMA) General Assembly, Helsinki, Finland, June 1964 and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975, 35th WMA General Assembly, Venice, Italy, October 1983, and the 41st WMA General Assembly, Hong Kong, September 1989, the 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996; 52nd WMA General Assembly, Edinburgh, Scotland, October 2000; 53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added); 55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added); and 59th WMA General Assembly, Seoul, October 2008.

### A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.
   The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of subjects, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my subject will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the subject's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.
7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
8. In medical practice and in medical research, most interventions involve risks and burdens.

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.
10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

## B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.
12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, Sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.
15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the Sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any SAEs. No change to the protocol may be made without consideration and approval by the committee.
16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on subjects or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects

must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.
25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.
27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.
28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.
29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious subjects, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.
30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

## C ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the subjects who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:
    - The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
    - Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the subjects who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.
33. At the conclusion of the study, subjects entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.
34. The physician must fully inform the subject which aspects of the care are related to the research. The refusal of a subject to participate in a study or the subject's decision to withdraw from the study must never interfere with the subject-physician relationship.
35. In the treatment of a subject, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the subject or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.