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 ID	NCT02476916
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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. 88 Sidney Street Cambridge, MA 02139-4169 USA Phone: + 1-617-649-8600 Fax: + 1-617-649-8618
EudraCT Number:	2015-000484-13
NCT Number:	NCT02476916
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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.

CCI [redacted]

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:

CCI [redacted]

PPD

PPD

Agios Pharmaceuticals, Inc.

Print/Sign/Date (dd mmm yyyy)

CCI [redacted]

PPD

PPD

Agios Pharmaceuticals, Inc.

Print/Sign/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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_____

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_____

Investigational site or name of institution and location (printed)

CCI

[redacted]

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 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).

CCI [redacted]

Extension Period

Primary Objective:

- Evaluate the long-term safety and tolerability of AG-348 administration in subjects with PK deficiency.

Secondary Objectives:

- 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.

CCI [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 8 years following the end of the Core Period.

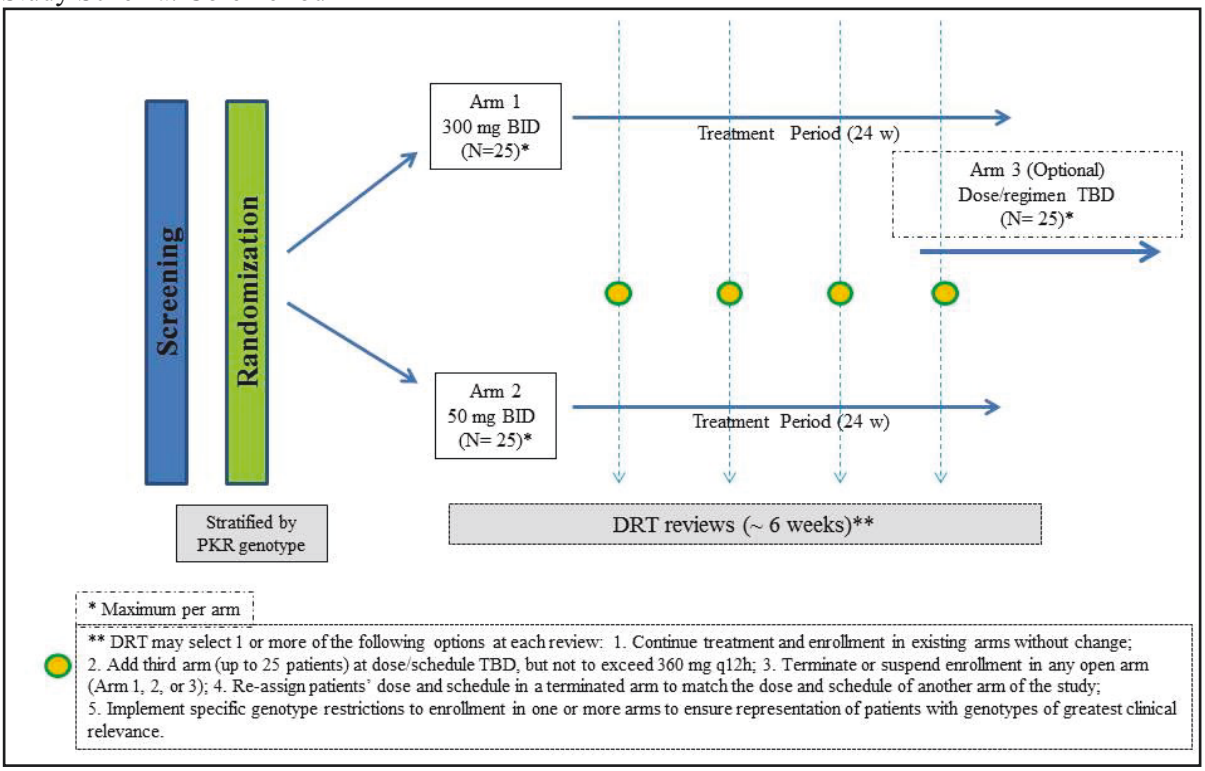
There are 2 different formulations of AG-348, capsules and tablets. While this study was initiated with the capsule formulation, the tablet formulation is planned to be introduced to gradually replace the capsule formulation by way of Amendment 6.

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; 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; and because this is the first study to evaluate AG-348 in subjects with PK deficiency, it is deemed important to focus closely on dose findings in this 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 be comprised of the study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer.

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 pharmacokinetic/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 periodic DRT meetings will not be required and will only occur ad hoc, as needed (appropriate monitoring and reporting of AEs will continue as required per protocol).

After subjects have completed the Core Period and entered the Extension Period, their pharmacokinetic/PD data will no longer be reviewed by the DRT.

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), the DRT will review cumulative safety data, available pharmacokinetic/PD data (Core Period only), 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.
- *Pharmacokinetic 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 pharmacokinetic/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 pharmacokinetic/PD sampling schedules as specified. There are no plans to implement a third dose arm because the Core Period is complete (as of 08 May 2017).

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 9.8.3).

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 DXA scans every 6 months through Month 30 and then annually.

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.

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 8 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 for up to 8 years after the end of the Core Period, except during the dose taper part of the Extension Period to optimize a subject's dose, during which weekly visits will be performed.

Subjects will also have a blood sample drawn for hematology assessments approximately 1 to 2 weeks following the subject's switch from the capsule to the tablet formulation. This sample may also be drawn away from the study site and sent to the central laboratory by qualified personnel (eg, home health care nurse).

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; the only exception is for subjects who transition to AG-348 treatment outside of Study AG348-C-003, as described in Section 10.1.

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 9.8.1 and Section 9.8.2.

Additional criteria for dose reduction will apply in the Extension Period, as detailed in Section 9.8.3.

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) ≥ 1.0×10^9/L.
   v. Platelet count ≥ 100×10^9/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 also be supplied as 5 mg, 20 mg, and 50 mg strength tablets to be administered orally.

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

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

Subjects will be switched from the capsule to the tablet formulation of AG-348 when the supply of the capsule formulation is exhausted or sooner as deemed appropriate by the Investigator and Medical Monitor.

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 8 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.

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 pharmacokinetic sampling as detailed in Appendix 1.3. The remainder of treated subjects will undergo limited pharmacokinetic 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.

Pharmacodynamics

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

The CCI [redacted] will only be conducted in clinical sites able to perform these assessments during the Core Period. 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. 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.

The only PD assessment in the Extension Period will be the measurement of PKR protein, which will be performed every 6 months up to (and including) Month 30. Appendix 1.2).

CCI [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.

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 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.

Pharmacokinetic/PD Analysis

Descriptive statistics will be used to summarize pharmacokinetic 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, pharmacokinetics, PD, and preliminary clinical activity data at regular intervals (approximately every 6 weeks during the Core Period) throughout the duration of the Core 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 periodic DRT meetings will not be required and will only occur ad hoc, as needed (appropriate monitoring and reporting of AEs will continue as required per protocol). When all the

subjects are in the Extension Period, pharmacokinetic/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

LIST OF TABLES

LIST OF FIGURES

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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
$AUC_{0\text{-}24hr}$	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
$AUC_{0\text{-}t}$	Area under the plasma concentration versus time curve from 0 to the last time point
BCRP	Breast cancer resistance protein
BID	Twice daily
BP	Blood pressure
BUN	Blood urea nitrogen
CBC	Complete blood count
C_{max}	Maximum (peak) 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
DDI	Drug-drug interaction

Abbreviation or Specialist Term	Definition
CYP	Cytochrome P450
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
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
LFT	Liver function test
MAD	Multiple ascending dose
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
mPKR	Pyruvate kinase isoform R mutants

Abbreviation or Specialist Term	Definition
NCI	National Cancer Institute
NOAEL	No-observed-adverse-effect-level
P-gp	P-glycoprotein
PCV13	Pneumococcal conjugate
PD	Pharmacodynamic
PEP	Phosphoenolpyruvate
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
UGT	Uridine 5′-diphospho-glucuronosyltransferase
ULN	Upper limit of normal
US	United States
VS	Vital signs
WBC	White blood cell

Abbreviation or Specialist Term	Definition
WMA	World Medical Association
WT	Wild-type

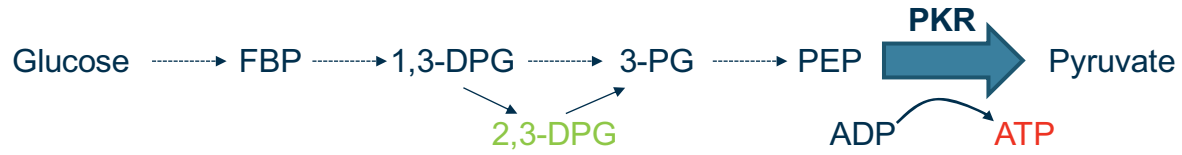
5. INTRODUCTION

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.

AG-348 (PYRUKYND) was approved by the US Food and Drug Administration (FDA) on 17 February 2022 for the treatment of hemolytic anemia in adults with PK deficiency (PYRUKYND (mitapivat) USPI, 2022).

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 pharmacokinetics 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 appeared to be 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 of CYP3A4 and CYP3A5 (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 appeared to be a weak inducer of human CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4/5, and UGT1A1 in vitro at clinically relevant concentrations.

In vitro studies using human liver microsomes and recombinant CYP enzymes have shown that AG-348 is primarily metabolized by CYP3A4 and CYP3A5, with minor contributions from CYP2C9, CYP2C8, and CYP1A2.

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. AG-348 did not demonstrate phototoxic potential in a GLP-compliant neutral red uptake phototoxicity assay in BALB/c 3T3 mouse fibroblasts.

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. A GLP-compliant combined fertility and early embryonic development study in rats demonstrated effects on maternal and paternal parameters, but no effects on mating or fertility parameters were observed. In a non–GLP-compliant 28-day range-finding toxicity study in juvenile rats, administration of AG-348 did not produce mortality, adverse clinical observations, differences in mean body weights, mean body weight gains, mean food consumption values, or macroscopic observations.

A GLP-compliant developmental and perinatal/postnatal reproduction study was conducted in Sprague Dawley rats. Administration of ≥50 mg/kg/day AG-348 was not tolerated in F0 generation females and resulted in unscheduled mortality due to dystocia/prolonged parturition. Maternal AG-348–related clinical observations were observed at ≥50 mg/kg/day. Increases in mean duration of gestation and percentage of dams with stillborn pups or no liveborn pups were observed only at 200 mg/kg/day. Reduced postpartum pup viability was observed at 200 mg/kg/day. Maternal doses ≤50 mg/kg/day did not result in postweaning F1 generation mortality, clinical observations, body weight, food consumption, or alterations on the day of preputial separation or vaginal opening. Maternal doses of AG-348 as high as 50 mg/kg/day did not affect learning and memory, mating and fertility, macroscopic observations, sperm evaluation, or any ovarian and uterine parameter in the F1 generation rats or naïve female rats mated with F1 generation males. For additional details of toxicology study findings, refer to the Investigator's Brochure.

5.2.2. Summary of Clinical Data

AG-348 has been evaluated in 2 completed clinical studies representing 72 healthy adult subjects, a single ascending dose (SAD) study (AG348-C-001) and a multiple ascending dose (MAD) study (AG348-C-002). Of these 72 subjects, 31 were 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 have been treated with AG-348.

5.2.2.1. Pharmacokinetics

The pharmacokinetics 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 pharmacokinetics 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 pharmacokinetics 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.

A capsule formulation of AG-348 was used in the SAD (AG348-C-001) and MAD (AG348-C-002) studies and is currently being used in this study (AG348-C-003). Phase 3 studies of AG-348 will be conducted with a tablet formulation. The same tablet formulation is planned to be introduced in this study by way of Amendment 6. In order to support the introduction of the tablet formulation, a phase 1, randomized, open-label, 2-period crossover relative bioavailability study (AG348-C-005) was conducted in healthy volunteers. The in-clinic phase of the study completed on 13 November 2017. Twenty-six subjects (13 male and 13 female) were enrolled in the study. One subject discontinued from the study after receiving a single 50 mg dose of the tablet formulation due to an adverse event (AE; head lice), which was assessed by the investigator and sponsor as not related to the study drug. The remaining 25 subjects (13 male and 12 female) who completed the study received a single 50 mg dose of the capsule formulation and a single 50 mg dose of the tablet formulation with a 7-day wash-out period between the 2 single-dose administrations. Systemic exposure to AG-348 was found to be similar between the 2 formulations, with a tablet to capsule AUC ratio of 1.05 and a maximum concentration (C_{max}) ratio of 1.19, suggesting similar relative bioavailability of the capsule and tablet formulations.

In Study AG348-C-012, an open-label, fixed-sequence study, the pharmacokinetics of AG-348 was evaluated in 14 healthy adult subjects in the presence and absence of itraconazole, a strong CYP3A4/5 and P-gp inhibitor, and in the presence and absence of rifampin, a strong CYP3A4/5 inducer. Systemic exposure of AG-348 increased in the presence of itraconazole compared with AG-348 alone, with the geometric mean area under the plasma concentration versus time curve from 0 to the last time point (AUC_{0-t}), $AUC_{0-\infty}$, and C_{max} ratios of AG-348 in the presence and absence of itraconazole being 4.7, 4.9, and 1.7, respectively. Systemic exposure of AG-348 in the presence of rifampin was lower compared with that of AG-348 alone, with the geometric mean AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} ratios of AG-348 in the presence and absence of rifampin being 0.09, 0.09, and 0.23, respectively.

Please refer to the Investigator's Brochure for a more detailed overview of available pharmacokinetic and pharmacodynamic data.

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 generally well tolerated among healthy adult subjects and adult subjects with PK deficiency. Two studies in healthy subjects, AG348-C-001 (SAD) and AG348-C-002 (MAD), have been completed. Safety data are available from 36 healthy subjects treated with AG-348 at single doses ranging from 30 to 2500 mg (SAD), from 36 healthy adult subjects treated with multiple doses ranging from 15 to 700 mg BID or 120 mg QD for 14 days (MAD). As of 27 March 2017, safety data from 52 subjects with PK deficiency randomized to treatment with 50 or 300 mg BID in this study (AG348-C-003) are also available.

Please refer to the Investigator's Brochure for a more detailed overview of available safety data.

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

After a single AG-348 dose, AEs 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 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 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.
- 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 periodic DRT meetings will not be required and will only occur ad hoc, as needed (appropriate monitoring and reporting of AEs will continue as described in Section 10.5.7).
- 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 drug-drug interactions (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.

- Liver function will be monitored with liver function tests (LFTs) every 3 months from Month 9 through Month 72 and every 6 months thereafter. As of Protocol Amendment 9, Version 10.0, transaminase increases are no longer considered an AESI for AG-348.

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 9.8).
- Adjusting the subject withdrawal criteria (Section 8.6).
- Terminating the trial.

5.2.4. Risks Associated with AG-348

Comprehensive information regarding the risks associated with AG-348 can be found in the Investigator's Brochure.

5.2.4.1. Risk of Acute 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. Potential Risk of Bone Mineral Density Decrease

AG-348 is a mild inhibitor of 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

As of Protocol Amendment 9, Version 10.0, transaminase increases are no longer considered a risk or AESI for AG-348.

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.

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 pharmacokinetics 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).

CCI

[redacted]

6.2. Extension Period

6.2.1. Primary Objective

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

- Evaluate the long-term safety and tolerability of AG-348 administration in subjects with PK deficiency.

6.2.2. Secondary Objectives

The secondary objectives of the Extension Period are to:

- 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.

CCI [redacted]

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 pharmacokinetic 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 pharmacokinetic sampling as detailed in Appendix 1.3. The remainder of treated subjects will undergo limited pharmacokinetic 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.
- Pharmacodynamic assessments during the Core Period will include 2,3-DPG, ATP (secondary objectives), CCI [redacted] will only be conducted in clinical sites able to perform these assessments during the Core Period.

  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.

CCI [redacted]

7. INVESTIGATIONAL PLAN

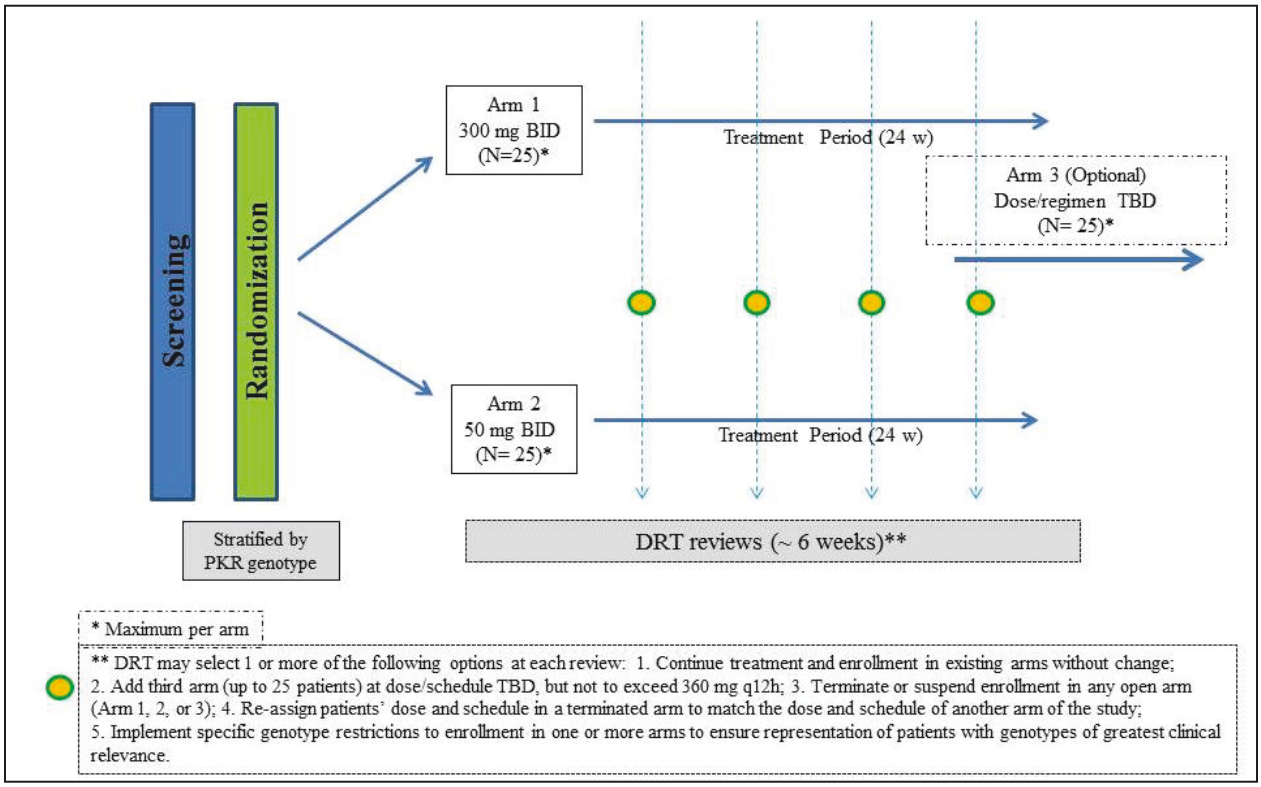
7.1. Overall Study Design

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 8 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; 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; and because this is the first study to evaluate AG-348 in subjects with PK deficiency, it is deemed important to focus closely on dose findings in this 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 be comprised of the study Coordinating Investigator, treating Investigators, Medical Monitor, Clinical Pharmacologist, Statistician, and Sponsor's Responsible Medical Officer.

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 pharmacokinetic/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 periodic DRT meetings will not be required and will only occur ad hoc, as needed (appropriate monitoring and reporting of AEs will continue as described in Section 10.5.7). After all subjects have completed the Core Period, their pharmacokinetic/PD data will no longer be reviewed by the DRT.

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), the DRT will review cumulative safety data, available pharmacokinetic/PD data (Core Period only), 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.
- *Pharmacokinetic 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 pharmacokinetic/PD sampling schedule in a potential third dosing arm will be determined by Sponsor's discretion and may follow either the extensive or limited pharmacokinetic/PD sampling schedules as specified. There are no plans to implement a third dose arm because the Core Period is complete (as of 08 May 2017).

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 9.8.3).

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 DXA scans every 6 months through Month 30 and then annually.

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 9.8.1 and Section 9.8.2.

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 8 years after the end of the Core Period, except during the dose taper part of the Extension Period to optimize a subject's dose during which weekly visits will be performed (see Section 9.8.3.1 for details).

There are 2 different formulations of AG-348, capsules and tablets. Studies AG348-C-001 and AG348-C-002 utilized the capsule formulation; however, all future planned studies will utilize the tablet formulation. While this study (AG348-C-003) was initiated with the capsule formulation, the tablet formulation is planned to be introduced to gradually replace the capsule formulation by way of Amendment 6.

Subjects will also have a blood sample drawn for hematology assessments approximately 1 to 2 weeks following the subject's switch from the capsule to the tablet formulation (Section 10.1). This sample may also be drawn away from the study site and sent to the central laboratory by qualified personnel (eg, home health care nurse).

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; the only exception is for subjects who transition to AG-348 treatment outside of Study AG348-C-003, as described in Section 10.1.

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.

Pharmacokinetic assessments will include serial blood sampling for pharmacokinetic profiles of AG-348 and its metabolite AGI-8702. There will be no pharmacokinetic sampling in the Extension Period.

Pharmacodynamic evaluations will include serial blood sampling for determination of levels of ATP and 2,3 DPG. Extensive pharmacokinetic/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 pharmacokinetic/PD sampling will be conducted on the remainder of treated subjects (Appendix 1.4).

CCI

[redacted]

7.2. Rationale for the Study Design

The primary and secondary objectives of this study are to evaluate the safety, tolerability, pharmacokinetics 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, pharmacokinetics 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, pharmacokinetics, 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 9.8.1 and Section 9.8.2). 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 pharmacokinetic 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 pharmacokinetic/PD relationship between AG-348 and the biomarkers ATP and 2,3-DPG, CCI [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, pharmacokinetics, 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 for subjects for whom AG-348 is safely tolerated and demonstrates clinical activity. The duration of the Extension Period is 8 years and is intended to ensure that subjects who are benefiting from AG-348 continue to have access to study drug. The Extension Period also serves to continue the evaluation of the long-term safety and tolerability of AG-348 in subjects with PK deficiency.

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.

7.3.5. Rationale for Dose Maintenance After Switch from AG-348 Capsules to Tablets

There are now 2 different formulations of AG-348, capsules and tablets. Studies AG348-C-001 and AG348-C-002 utilized the capsule formulation; however, all future planned studies will utilize the tablet formulation. While this study (AG348-C-003) was initiated with the capsule formulation, the tablet formulation is planned to be introduced to gradually replace the capsule formulation by way of Amendment 6. In order to support the introduction of the tablet formulation, Study AG348 C-005 was performed in healthy subjects to evaluate the relative bioavailability of the tablet and capsule formulation. Preliminary results from this study (refer to Section 5.2.2.1 for study details) suggest that exposures, defined as $AUC_{0-\infty}$, AUC_{0-t}, and C_{max},

between tablet and capsule formulations are similar. Therefore, no dose adjustment is required following a switch to the tablet formulation. Based on these results, when subjects are switched from AG-348 capsules to tablets, the same dose of tablet as the capsule dose should be administered.

7.4. Modifications Allowed During Declared Public Health Emergencies and Natural Disasters

In the event of a declared public health emergency or natural disaster that affects a geographic area (eg, state, province, country, region, continent) and impedes adherence to protocol-specified procedures, certain modifications (Section 7.4.1) are allowable, when consistent with applicable regulations and guidance, to ensure subject safety, maintain compliance with good clinical practice (GCP), and minimize risks to trial integrity; the protocol must be followed to the fullest extent possible. These modifications are allowable only for the duration of the declared public health emergency or natural disaster, including any renewals of the declaration. During this period, the need for all implemented modifications will be reassessed when warranted as the situation evolves. Examples of declared public health emergencies and disasters are:

- The public health emergency related to coronavirus disease 2019 (COVID-19) declared by the US Secretary of Health and Human Services in 2020
- The Australian Bushfires Disaster declared by the Australian Attorney-General in 2020

Documented approval from the Agios Medical Monitor (or designee) is required before these modifications can be implemented.

7.4.1. Allowable Modifications

The following modifications are allowed in the event of a public health emergency or natural disaster and must be reported as protocol deviations; refer to Appendix 1 for the timing of assessments:

- Alternative distribution of study drug
  - A 3-month supply of AG-348 may be shipped to a local health care provider or pharmacy or, if necessary, directly to a subject. Delivery of a greater than 3-month supply of AG-348 must be reviewed and approved in advance by the Medical Monitor, in agreement with the investigator.
  - Secure, trackable delivery methods (delivery service companies [eg, DHL], couriers, and hand delivery) must be used.
  - Medical Monitor approval is required before each shipment. Shipment will be permitted only if, at minimum, a telemedicine visit has been conducted that incorporates appropriate safety assessments.
- Returning unused study drug and empty study drug packaging
  - Return of unused study drug and empty study drug packaging may be delayed until the subject's next visit to the study site. In certain circumstances, the nature

of the return process may vary (eg, personal protective equipment may be required).

- Telemedicine visits
  - Telemedicine visits, preferably via video conference, are permissible for all assessments that can be completed via this mode (eg, medical history, concomitant medications, review of AEs).
- Use of laboratories and health care providers not specified in the clinical trial documentation
  - For assessments that cannot be completed via telemedicine, the use of health care providers and laboratories that are not specified in the clinical trial documentation (such as an imaging facility, clinic, or local practice that is more readily accessible by the subject) is permissible for all assessments that can be completed via this mode (eg, blood collection for laboratory assessments, ECG, physical examinations, imaging).
  - Use of a laboratory or health care provider not specified in the clinical trial documentation requires coordination between the subject, the investigator, and the subject's local health care provider.
  - The investigator must document their review of the results provided by laboratories and health care providers not specified in the clinical trial documentation.
- Home health study support
  - For assessments that cannot be completed via telemedicine, home health nursing visits are permissible for all assessments that can be completed via this mode (eg, physical examination, collection of laboratory samples).
  - The Sponsor may facilitate and coordinate these visits with the study site.
  - The Investigator must document their review of the results of home health nursing visits.
- Virtual informed consent/reconsent in lieu of in-person informed consent/reconsent
  - Reconsent (ie, consenting to an amended version of the protocol or to participate in the Extension Period of the study) may be completed virtually and documented in the relevant subject medical records.
  - The other allowable modifications described in this section may require consent from the subject because their implementation requires a variation from the specifications in the protocol to which the subject has consented (eg, consent for a home visit, consent to provide name and address to a third-party delivery service, consent to a new mode of completing study procedures and receiving study drug). In these instances, consent may be completed virtually and documented in the relevant subject medical records.

7.5. 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 or business 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 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.

2. 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.

3. Have Hb ≤ 12.0 g/dL (if male) or ≤ 11.0 g/dL (if female).
4. 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.
5. 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.
6. Have Eastern Cooperative Oncology Group (ECOG) Performance Status ≤ 2.
7. 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.
8. 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) ≥ 1.0×10^9/L.

v. Platelet count ≥ 100 × 10^9/L.

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

9. 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.

10. 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 may 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 9.12.2.1)
- Investigator decision
- Protocol violation (nonadherence to protocol requirements)
- Lost to follow-up
- Approved drug available for indication
- Other

Subjects in the Extension Period who have not experienced a robust and sustained increase in hemoglobin (see Section 9.8) will be discontinued from the study.

Pregnancy will not be considered a reason for subjects' withdrawal from the study. Should a subject become pregnant during the study, the pregnancy should be reported to the Sponsor as detailed in Section 11.3, and the subject should suspend treatment with AG-348 immediately. Abrupt discontinuation of AG-348 dosing may result in withdrawal hemolysis; subjects should be monitored closely for signs of hemolysis and worsening or recurrence of anemia.

Following discussion with the Medical Monitor, or Sponsor designee, the pregnant subject may be offered the opportunity to remain on the study with study drug suspended while pregnant or breastfeeding. If the subject remains on the study, the subject will be expected to continue with all other aspects of study participation with the exception of DXA scans. Following completion of pregnancy and/or breastfeeding, the subject who wants to resume treatment should go to the site for an unscheduled visit and undergo assessments to determine if the subject can resume treatment. Suggested assessments are physical examination (including weight), vital signs, hematology, LFTs, hormonal testing, iron panel, haptoglobin, lipids, and DXA. Following this visit, the Investigator should discuss with the Medical Monitor the appropriateness of the subject resuming treatment and the dose at which the subject should resume.

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. AG-348 will also be supplied as 5 mg, 20 mg, and 50 mg strength tablets to be administered orally.

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 or tablet strength should the evolving trial experience demonstrate that the specific capsule or tablet strength fills no additional need beyond the other capsule or tablet 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 and tablets 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. Switch to Tablet Formulation of AG-348

By way of Amendment 6, subjects will be switched from the capsule to the tablet formulation of AG-348 when the supply of the capsule formulation is exhausted, or sooner as deemed appropriate by the Investigator and the Sponsor. Subjects will undergo a hematology assessment after switching formulations (refer to Section 10.1).

9.4. 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, member of the staff specifically authorized by the Investigator, or party designated to deliver study drug directly to subjects (if acceptable by local regulations).

9.5. 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).

9.6. Blinding

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

9.7. 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 9.10).

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 pharmacokinetic/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 pharmacokinetic/PD blood draws.

Subjects receiving limited pharmacokinetic/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 pharmacokinetic/PD blood draws.

Subjects receiving extensive pharmacokinetic/PD sampling on Day 1 and 15 will also have limited pharmacokinetic/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 pharmacokinetic/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 pharmacokinetic/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/tablets whole and to not chew the capsules/tablets. For subjects who have difficulty swallowing capsules/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).

9.8. 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 will be discontinued from the study (Section 8.6). For this purpose, a robust and sustained increase in hemoglobin is defined as an increase of at least 1.0 g/dL from baseline for ≥3 of the last 4 regularly scheduled hemoglobin analyses performed by the central laboratory that were collected in the absence of transfusion (ie, no transfusions in the period from the first to the last of these hemoglobin analyses and in the 2 months prior to the first hemoglobin analysis).

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. A dose-taper regimen for discontinuation of study drug is provided in Section 9.8.5.

9.8.1. 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 9.8).

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; Contact the Medical Monitor (or designee) to discuss specific cases that may need to be managed as Grade 3 events (see below).
Grade 3	Discontinue dosing. Consider performing a dose taper to discontinue dosing. Dose tapers should be used after careful consideration of the relative risk of maintaining a subject on their current dose of AG-348 versus decreasing the dose of AG-348. 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). 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, using a dose taper, 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.

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).

9.8.2. 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.

9.8.3. Individual Optimal Maintenance Dose-Finding in Extension Period

9.8.3.1. Gradual Dose-Taper Regimen in Extension Period

As of Amendment 5 of the protocol, 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.

Subjects who did not undergo the dose taper in the Extension Period when it was implemented under Amendment 5 of the protocol are encouraged to do so under Amendment 6.

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 samples for weekly Hb monitoring during the gradual dose-taper regimen may be drawn away from the study site and sent to the central laboratory by qualified personnel (eg, home health care nurse).

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 9.8.3.1. 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 drawn away from the study site and sent to the central laboratory by qualified personnel (eg, home health care nurse).

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.

9.8.4. 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 9.8.1). Other reasons for treatment termination are provided in Section 8.6.

9.8.5. Dose Taper for Discontinuation of Study Drug

Subjects who discontinue the study drug early or successfully complete the Extension Period will undergo a dose-taper regimen so as not to abruptly stop AG-348 treatment, thereby potentially risking withdrawal hemolysis. For subjects on a dose higher than 50 mg BID, the following sequential steps should be used (each step of the dose taper should last approximately 7 days):

- 300 mg BID
- 200 mg BID
- 100 mg BID
- 50 mg BID

For subjects at 50 mg BID or a lower BID dose either at the start of the taper or at the end of the sequential steps above, the dose taper regimen in Table 5 should be followed.

Table 5: Dose Taper Regimen for Discontinuation of Study Drug, Subjects Receiving AG-348 at a Dose of 50 mg BID or Lower

Starting Dose	First Step ×7 days	Second Step ×7 days
50 mg BID	50 mg QD	20 mg QD
25 mg BID	25 mg QD	5 mg QD
20 mg BID	20 mg QD	5 mg QD
5 mg BID	5 mg QD	n/a

Abbreviations: BID = twice daily; n/a = not applicable; QD = once daily.

For subjects who are at a dose that is not listed above or in Table 5, the Medical Monitor should be contacted for dose-taper guidance.

9.9. 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 8 years following completion of the Core Period.

9.10. 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.

9.11. 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 to the site and to the subject, 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. 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.

Study drug is expected to be dispensed to the subject at the study site during scheduled on-site study visits; under exceptional circumstances and with agreement of the Sponsor (or representative), study drug that was intended to be dispensed during scheduled on-site study visits can be provided to a subject's home, if acceptable by practice and allowed by local regulations. For scheduled telemedicine study visits (See Appendix 1.2), study drug will be shipped to the subject's home by the Investigator, member of the staff specifically authorized by the Investigator, or party designated to deliver study drug, if acceptable by practice and allowed by local regulations.

All unused study drug must be properly disposed of in compliance with local procedures and governing regulations. Documentation of the method of destruction should be maintained in the Investigator's files.

9.12. Prior and Concomitant Medications and Treatments

9.12.1. 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.

9.12.2. 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 and CYP3A5, with minor contributions from CYP2C9, CYP2C8, and CYP1A2. In addition, AG-348 has been shown to be a weak time-dependent CYP3A4/5 inhibitor and a potential inducer of CYP3A4 and UGT1A1 in vitro. Based on these results, below is a list of concomitant therapy to be avoided and concomitant therapy requiring careful monitoring.

9.12.2.1. Concomitant Therapy to be Avoided (Prohibited)

The following therapies are to be avoided during the 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).
- 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 should be 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.

As of Protocol Amendment 8, v9.0, the above list of prohibited therapies was modified such that digoxin and strong inhibitors of P-gp were no longer prohibited (based on PBPK simulations that suggested there is no risk of DDI between these drugs and AG-348); however no changes were made to the eligibility criteria (Section 8.3.1), because enrollment had completed prior to finalization of Protocol Amendment 8, v9.0.

9.12.2.2. Concomitant Therapy Requiring Careful Monitoring (Use with Caution)

The following therapies should be replaced with alternative treatments. If this is not possible, subjects receiving these drugs should be appropriately 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).
- Moderate inducers of CYP3A4 (listed in Appendix 4.2)
- Proton-pump inhibitors and H2-receptor antagonists (listed in Appendix 4.5). 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 until 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).
- Deferoxamine, deferasirox, and deferiprone. AG-348, as a potential UGT1A1 inducer, has the potential to reduce the effectiveness of iron chelators metabolized by UGT1A1.

9.12.2.3. 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 comedication of oral penicillin is 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.

The Sponsor has conducted a risk assessment for concomitant use of a COVID-19 vaccine with AG-348 with specific consideration for the trial population and determined that the COVID-19 vaccine given to a trial subject is considered a simple concomitant medication with no interaction that requires advice on timing of the vaccine or other aspects that need to be mitigated.

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.

9.13. 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.
- 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. At scheduled telemedicine visits (every 6 months starting at Month 75; Appendix 1.2), assessments will be collected from subjects remotely by the Investigator or site staff; for sites where telemedicine is not permitted by local regulations, subjects are to complete their assessments in-person at the site. 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; the only exception is for subjects who transition to AG-348 treatment outside of Study AG348-C-003, as described below. A serum or urine pregnancy test 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 female subjects of childbearing potential, and any pregnancies must be reported (see Section 11.3).

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 6 summarizes the details of the re-scheduling of these assessments as described in Appendix 1.1.

Approximately 1 to 2 weeks after switching to the tablet formulation, subjects will undergo a hematology assessment. If Hb increases or decreases by >1.0 g/dL from the subject's most recent

value prior to switching formulations, the hematology assessment should be repeated and the Medical Monitor should be contacted.

During the Extension Period up to Month 30, all scheduled visits, with the exception of blood draws for those subjects undergoing gradual dose-taper regimen (Section 9.8.3) and blood draws for those who have switched from the capsule to the tablet, must be conducted by the Investigator and at the participating authorized investigative site; local primary care visits will not be allowed.

Table 6: 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; pharmacokinetics/PD

Abbreviations: ECG = electrocardiogram; EPO = erythropoietin; PD = 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 pharmacokinetic 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 pharmacokinetic/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.

Subjects may transition to receiving treatment with mitapivat (the international nonproprietary name of AG-348) outside of Study AG348-C-003 (eg, commercial) and may follow an altered schedule of assessments depending on whether they complete the study or prematurely discontinue, as described below.

- Subjects who complete study (ie, complete the last scheduled visit) before transitioning:

  - Subjects who intend to complete the study and immediately transition to receiving treatment with mitapivat outside of Study AG348-C-003 without interruption of mitapivat dosing may have their End of Study visit at Month 102. These subjects would not be dispensed study drug on Month 102, not be required to undergo the final dose taper, and not attend the Follow-up Visit.

- Subjects who prematurely discontinue study before transitioning
  - Subjects who transition to receiving treatment with mitapivat outside of Study AG348-C-003 before their Month 102 Visit will attend an End of Study visit that includes the same assessments that would have been performed on Month 102 and will be withdrawn from the study. These subjects will not be dispensed study drug at their End of Study visit and will not attend the Follow-up Visit. Subjects who intend to immediately transition to receiving treatment with mitapivat outside of Study AG348-C-003 without interruption of mitapivat dosing are not required to undergo the final dose taper.

As summarized in Section 9.8, withdrawal hemolysis is an identified risk of mitapivat, and subjects should undergo dose taper if treatment with mitapivat is to be discontinued or interrupted. For subjects who transition to receive treatment with mitapivat outside of Study AG348-C-003, please refer to the dose management instructions (eg, package insert) to assess the need for subjects to undergo dose taper during the transition.

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 Mayo Medical Laboratories (Rochester, MN) or any participating investigative site's local hematology laboratory. PKR genotyping will be conducted at Centogene AG (Rostock, Germany).

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 up to Month 30; a complete physical examination will be performed at Month 30. After Month 30, a focused physical examination will be conducted at the End of Study Visit.

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.

As of Protocol Amendment 9, Version 10.0, ECGs are no longer required to be assessed after the Month 30 visit; however, ECGs may be performed at the discretion of the Investigator when clinically indicated.

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. Subjects in the Extension Period will have DXA scans every 6 months through Month 30 and then annually 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. These parameters will be assessed at time points according to the Schedules of Assessments (Appendix 1).

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 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. 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 and Assessments of Childbearing Potential

Menstruating female subjects will be required to fill out a paper-based menstrual cycle diary, which 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.

After Month 30, female subjects will no longer fill out the menstrual cycle diary. Subjects should return the diary to the site at or before the End of Study visit.

Starting at Month 33, changes in childbearing potential of female subjects (as defined in Appendix 6) will be recorded in the source documents and eCRF, including the date of the change.

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 pharmacokinetic sampling as detailed in Appendix 1.3. The remainder of treated subjects will undergo limited pharmacokinetic 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, pharmacokinetic 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 6.)

Pharmacokinetic samples will not be collected during the Extension Period.

The collection times for postdose pharmacokinetic samples will start from the time that dosing is completed; eg, a pharmacokinetic 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.

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. CCI [redacted] will be drawn every other study visit (every 6 months, up to and including Month 30; 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 6).

CCI [redacted]

- AG-348 target engagement and stimulation of glycolytic pathway 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).
3.
4. Pharmacokinetics.
5.
6.

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

As of Protocol Amendment 9, Version 10.0, transaminase increases are no longer considered an AESI for AG-348 and will no longer be reported as AESIs.

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

As of Protocol Amendment 9, Version 10.0, there are no AEs considered to be AESIs.

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

As of Protocol Amendment 9, Version 10.0, transaminase increases are no longer considered an AESI for AG-348 and will no longer be reported as AESIs. The following procedures in this section are no longer to be performed.

In the event of a transaminase increase of >2.5 × baseline or an increase in AST or ALT to Grade ≥2 in severity, whichever is lower, the study site should report this occurrence to the Sponsor, using the AESI page in the eCRF, within 24 hours of their first knowledge of the event.

An LFT panel should then be performed weekly until the transaminases have decreased to <2.5 × baseline. Additionally, the following tests should be performed to gain further information on the possible cause of the transaminase increase:

1. Rule out biliary obstruction by liver imaging (liver CT scan, liver MRI, liver ultrasound, or magnetic resonance cholangiopancreatography, as clinically indicated).
2. Viral screen for Epstein-Barr virus (EBV), cytomegalovirus (CMV) Abs, Hepatitis A Ab, HBsAg, HCVAb (with an RT-PCR [reverse transcriptase-polymerase chain reaction] test performed if HCVAb is positive), HIV-1Ab, and HIV-2Ab.
3. Autoimmune hepatitis panel consisting of the following: serum antinuclear antibody, antismooth muscle antibody, liver-kidney microsomal type 1, antibody to soluble liver antigen, and antimitochondrial antibody when transaminase increase meets the criteria of AESI and repeated 4 weeks later, if the results were negative the first time.

The Investigator should refer to Section 9.8.1 to determine if a dose adjustment is needed. If the Investigator is not sure whether or not a dose adjustment is needed, they should consult with the Medical Monitor or designee.

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.

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.

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 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 24 hours 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 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 female subjects of childbearing potential 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 lifestyle 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 7 provides the probability within a dose arm of detecting 1 or more AEs with varying sample size and the true underlying AE rates.

Table 7: 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 provided 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.

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 MedDRA and the incidence of AEs occurring in the treatment-emergent period (new or worsening from baseline) will be summarized by primary MedDRA 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. Pharmacokinetic/PD Analysis

CCI [redacted]

12.5.1. Pharmacokinetic Analysis

Descriptive statistics will be used to summarize pharmacokinetic 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 pharmacokinetic 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.

CCI [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, pharmacokinetics, PD, and preliminary clinical activity data at regular intervals (approximately every 6 weeks during the Core Period) throughout the duration of the Core Period; no pharmacokinetic/PD data will be reviewed 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 periodic DRT meetings will not be required and will only occur ad hoc, as needed (appropriate monitoring and reporting of AEs will continue as described in Section 10.5.7).

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, pharmacokinetics, PD, and preliminary clinical activity (eg, changes in Hb levels). When all the subjects are in the Extension Period, pharmacokinetic/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 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 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: 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: **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		
Pharmacokinetic 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	
CCI													
CCI													
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: 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 deficiency = pyruvate kinase deficiency; PPD 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, pharmacokinetic/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 pharmacokinetic/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.

   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 (CO_2) 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 pharmacokinetic/PD sampling will be conducted on Days 1 and 15 (see for details), followed by limited pharmacokinetic/PD sampling from Week 3 to Week 24 (see Appendix 1.3). Limited pharmacokinetic/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 pharmacokinetic and PD assessments, respectively, and refer to Section 10.9 for sample processing and storage guidelines.
30. A single nontime critical sample will be collected. Laboratory testing may also include assessment of CCI [redacted] (see Section 10.7)
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

Schedule of Assessments: Extension Period – Month 9 through Month 30

Visit: Assessment(s)	Month 9	Month 12	Month 15	Month 18	Month 21	Month 24	Month 27	Month 30
Approximate Study Day	**259**	**349**	**439**	**529**	**619**	**709**	**799**	**889**
Visit Window	**± 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
ECOG Performance Status	X	X	X	X	X	X	X	X
Vital signs (BP, HR, RR, T)[2]	X	X	X	X	X	X	X	X
12-lead ECG[3]		X		X		X		X
DXA scan		X		X		X		X
Laboratory evaluations[4]								
Hematology (CBC)[5]	X	X	X	X	X	X	X	X
Haptoglobin		X		X		X		X
EPO levels[6]		X		X		X		X
Hepcidin		X		X		X		X
Serum chemistry[7]	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
Urinalysis[11]	X	X	X	X	X	X	X	X
Lipids[12]	X	X	X	X	X	X	X	X
Hormonal testing[13]	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
Study drug dispensed[15]	X	X	X	X	X	X	X	X
PD assessments (PKR protein)		X		X		X		X
Dispense/collect menstrual cycle diary[16]	X	X	X	X	X	X	X	X
Adverse events[17]	Continuous							
Transfusion record[18]	X	X	X	X	X	X	X	X
Concomitant medications/procedures	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 deficiency = pyruvate kinase deficiency; CCI [redacted] 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.

Subjects will have a blood sample drawn for hematology assessments approximately 1 to 2 weeks following the subject's switch from the capsule to the tablet formulation of AG-348. This sample may be drawn away from the study site and sent to the central laboratory by qualified personnel (eg, home health care nurse).

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 (CO_2) 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. 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 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).
16. 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.
17. 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.
18. All transfusions must be recorded in the eCRF.

Schedule of Assessments: Extension Period – Month 33 through Month 102 and Follow-up

Visit: **Assessment(s)**	**Month** **33** **45** **57** **69**	**Month** **36** **48** **60** **72**	**Month** **39** **51** **63**	**Month** **42** **54** **66**	**Month** **75** **87** **99** **(Telemedicine[2])**	**Month** **78** **90**	**Month** **81** **93** **(Telemedicine[2])**	**Month** **84** **96**	**Month** **102[1]**	**Follow-up[1]**
Approximate Study Day	**979** **1339** **1699** **2059**	**1069** **1429** **1789** **2149**	**1159** **1519** **1879**	**1249** **1609** **1969**	**2239** **2599** **2959**	**2329** **2689**	**2419** **2779**	**2509** **2869**	**3049**	**3079**
Visit Window	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**
Physical examination/weight[3]									X	X
Vital signs (BP, HR, RR, T)[4]		X		X		X		X	X	X
DXA scan[1]				X		X			X	X (ED only)
Childbearing potential[5]	X									
Laboratory evaluations										
Hematology (CBC)[6]	X	X	X	X		X		X	X	X
Haptoglobin	X	X	X	X		X		X	X	X
Liver function tests[7]	X	X	X	X		X		X	X	X
Iron panel[8]		X		X		X		X	X	X (ED only)
Lipids[9]				X		X			X	X
Hormonal testing[10]	X	X	X	X		X		X	X	X
Study drug administration	X									
Study drug dispensed[11]	X	X	X	X	X	X	X	X	X[1]	
Adverse events[12]	Continuous									X
Transfusion record[13]		X		X		X		X	X	X
Concomitant medications/procedures	X	X	X	X	X	X	X	X	X	X

Abbreviations: ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase, BP = blood pressure; CBC= complete blood count; DXA = dual-energy x-ray absorptiometry; ED = early discontinuation; HR = heart rate; 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 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.
Shaded columns represent visits that may be conducted away from the study site and shipped to the central laboratory by qualified personnel (eg, home health care nurse).
Subjects will have a blood sample drawn for hematology assessments approximately 1 to 2 weeks following the subject's switch from the capsule to the tablet formulation of AG-348. This sample may also be drawn away from the study site and sent to the central laboratory by qualified personnel (eg, home health care nurse).

1. Subjects who intend to complete the study and immediately transition to receiving treatment with mitapivat outside of Study AG348-C-003 without interruption of mitapivat dosing may have their End of Study visit at Month 102. Subjects who transition to receiving treatment with mitapivat outside of Study AG348-C-003 before their Month 102

Visit will attend an End of Study visit that includes the same assessments that would have been performed on Month 102 and will be withdrawn from the study. These subjects would not be dispensed study drug at the End of Study visit and not attend the Follow-up Visit. Subjects who intend to immediately transition to receiving treatment with mitapivat outside of Study AG348-C-003 without interruption of mitapivat dosing are not required to undergo the final dose taper. Dual-energy X-ray absorptiometry scans are not required to be performed at the End of Study visit if it was performed within the past 6 months.

2. Telemedicine visits will be conducted via phone or video call to assess current supply of study drug, assess changes in underlying health status, and record AEs and concomitant medications. Investigators should use prudent medical judgment to determine whether to request for subjects to attend unscheduled on-site visits based on information gathered at a telemedicine visit. For sites where telemedicine is not permitted by local regulations, subjects are to complete their assessments in-person at the site.
3. Focused physical examination; genital and rectal examinations will be performed at the discretion of the Investigator.
4. Vital signs will include systolic and diastolic blood pressure, heart rate, respiratory rate, and temperature.
5. Changes in childbearing potential of female subjects (as defined in Appendix 6) will be recorded, including the date of the change.
6. 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.
7. ALP, ALT, AST, total bilirubin, and indirect bilirubin
8. Iron, total iron-binding capacity (TIBC), transferrin saturation, and ferritin will be performed prior to dosing.
9. Total cholesterol, HDL-C, and triglyceride samples will be collected.
10. Serum estrone, estradiol, and free and total testosterone are to be collected in the morning, following an overnight fast.
11. Study drug will be dispensed during scheduled on-site study visits or shipped to the subject's home for telemedicine visits on a 3-month schedule. Subjects for whom visits represented by shaded columns are performed away from the study site may receive their drug supply from a home health care nurse. For scheduled telemedicine study visits, study drug will be shipped to the subject's home if acceptable by practice and allowed by local regulations. The amount of study drug provided to subjects 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).
12. 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.
13. All transfusions must be recorded in the eCRF.

Appendix 1.3. Schedule of Assessments: Extensive Pharmacokinetic/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]
Pharmacokinetic 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
CCI [redacted]														

Abbreviations: 2,3 DPG = 2,3 diphosphoglycerate; ATP = adenosine triphosphate; D = day; DPG = diphosphoglycerate; hr = hours; min = minutes; PD = pharmacodynamic; PPD [redacted]; W = week.

1. The acceptable time window will be within 60 minutes prior to study treatment dose administration for the predose pharmacokinetic/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 pharmacokinetic/PD samples.
3. The acceptable time window will be within ± 30 minutes of the scheduled collection time for the 4, 8, and 12-hour pharmacokinetic/PD samples.
4. To be collected on Day 1 only.
5. CCI [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. CCI [redacted]

Appendix 1.4. Schedule of Assessments: Limited Pharmacokinetic/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: **Assessment(s)**	Predose[1]	Predose[1]	Predose[1]	Predose[1]	Predose[1]	Predose[1]	Predose[1]	Predose[1]	Predose[1]
Pharmacokinetic blood sample	X	X	X	X	X	X	X	X	X
2,3 DPG/ATP	X	X	X	X	X	X	X	X	X

CCI [redacted]

Abbreviations: 2,3 DPG = 2,3 diphosphoglycerate; ATP = adenosine triphosphate; D = day; DPG = diphosphoglycerate; hr = hours; min = minutes; PD = pharmacodynamic; CCI [redacted] W = week.

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

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

Abbreviations: AUC = area under the plasma concentration versus time curve; CYP = cytochrome P450.
This list is not comprehensive. Medications on this table have the potential to be removed, or new medications may be added when new information is available. Refer to University of Washington DDI database for the most up-to-date list.
Strong Inhibitor = >5-fold increase in AUC.

1. Moderate Inhibitor = >2-fold, < 5-fold increase in AUC.Although grapefruit is classified as a moderate CYP3A4 inhibitor, it is prohibited, as is grapefruit juice.

Appendix 4.2. Strong and Moderate CYP3A4 Inducers

Strong CYP3A4 Inducers: Prohibited (To be Avoided)	Moderate CYP3A4 Inducers: Substitute or Use with Caution
Carbamazepine	Semagacestat
Oxcarbazepine	Efavirenz
Phenobarbital	Tipranavir And Ritonavir
Phenytoin	Dabrafenib
Rifabutin	Lesinurad
Rifampin	Bosentan
St. John's Wort	Thioridazine
	Rifabutin
	Lorlatinib
	Nafcillin
	Talviraline
	Lopinavir
	Daclatasvir
	Asunaprevir
	Beclabuvir
	Modafinil
	PF-06282999
	Etravirine
	Elagolix
	Lersivirine
	Telotristat Ethyl

Abbreviation: CYP = cytochrome P450.
Note: Corticosteroids may induce CYP3A4. Although the use of corticosteroids is not prohibited, their use should be minimized as much as is medically feasible.
This list is not comprehensive. Medications in this table have the potential to be removed, and new medications may be added when new information is available. Refer to the University of Washington Drug Interaction Database for the most up to date list.

Appendix 4.3. Strong P-glycoprotein Inhibitors (Allowed)

As of Protocol Amendment 8, v9.0, strong inhibitors of P-gp were no longer prohibited; however, no changes were made to the eligibility criteria (Section 8.3.1), because enrollment had completed prior to finalization of Protocol Amendment 8, v9.0.

Appendix 4.4. 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

Abbreviations: HMG CoA = β-Hydroxy β-methylglutaryl-CoA; LAAM = levo-alpha-acetylmethadol; TMU = 1,3,7-trimethyluric acid.
This list is not comprehensive. Medications in this table have the potential to be removed, and new medications may be added when new information is available. Refer to the University of Washington Drug Interaction Database for the most up-to-date list

Appendix 4.5. Proton-Pump Inhibitors and Histamine 2-Receptor Antagonists (Substitute or Use With Caution)

Proton-pump inhibitors:	
Dexlansoprazole	Omeprazole
Esomeprazole	Rabeprazole
Lansoprazole	Pantoprazole
Histamine 2-Receptor antagonists:	
Cimetidine	Nizatidine
Famotidine	Ranitidine

This list is not comprehensive. Medications in this table have the potential to be removed, and new medications may be added when new information is available. Refer to the University of Washington Drug Interaction Database for the most up to date list.

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.

APPENDIX 6: DEFINITION OF WOMEN OF CHILDBEARING POTENTIAL

A woman is considered fertile after menarche and until becoming postmenopausal unless permanently sterile. If fertility is unclear (eg, amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before administration of the first dose of study drug, additional evaluation should be considered.

A woman is not considered a woman of childbearing potential if any of the following apply:

1. Premenarchal
2. Premenopausal female with 1 of the following:

   - Documented hysterectomy
   - Documented bilateral salpingectomy
   - Documented bilateral oophorectomy

   For individuals with permanent infertility due to an alternate medical cause other than the above (eg, Mullerian agenesis, androgen insensitivity), Investigator discretion should be applied to determining study eligibility.

   Documentation can come from the subject's medical records, medical examination, or medical history interview.
3. Postmenopausal female

   - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
     – A high FSH level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormone replacement therapy; however, in the absence of 12 months of amenorrhea, confirmation with >1 FSH measurement is required.

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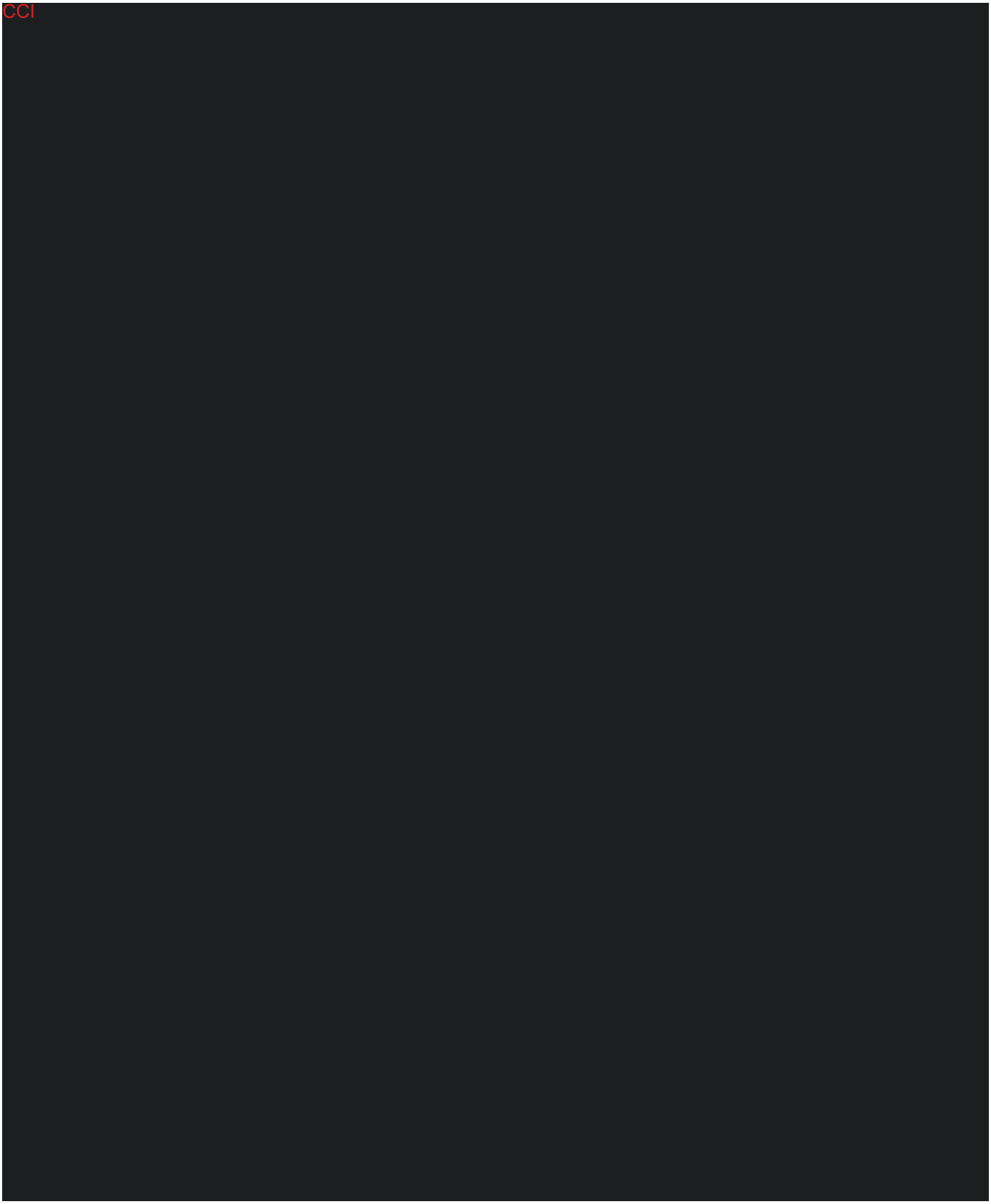

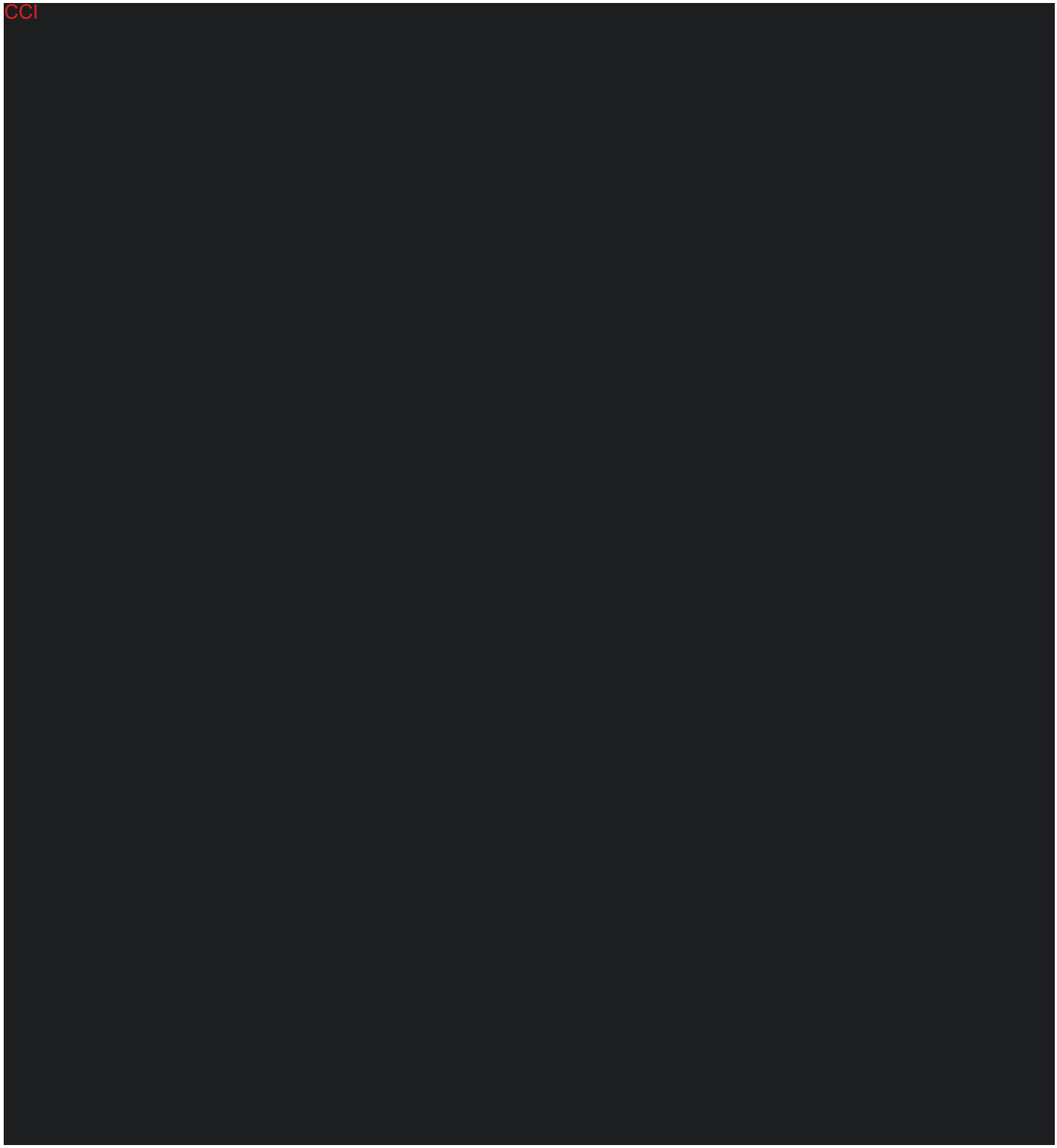
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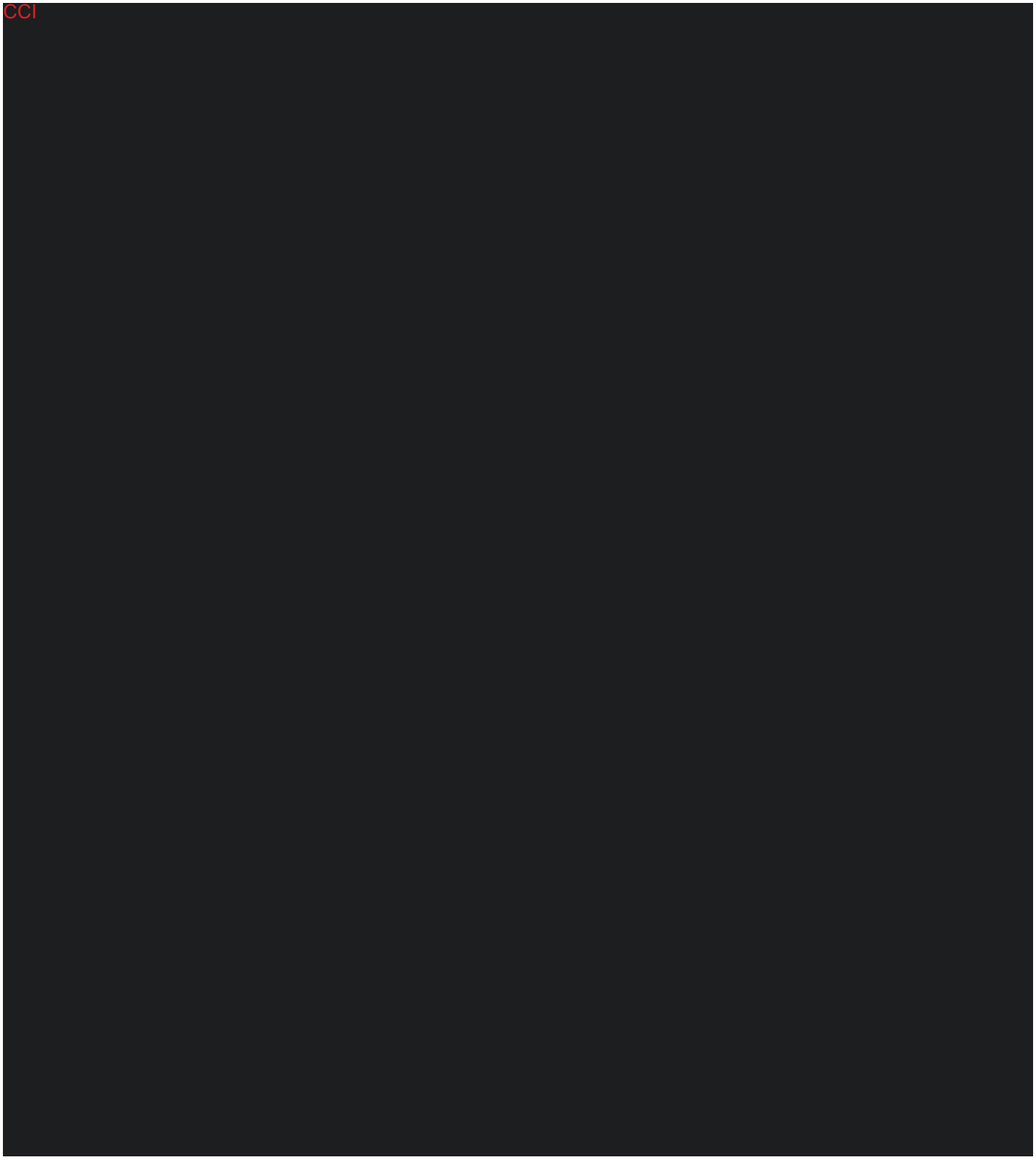

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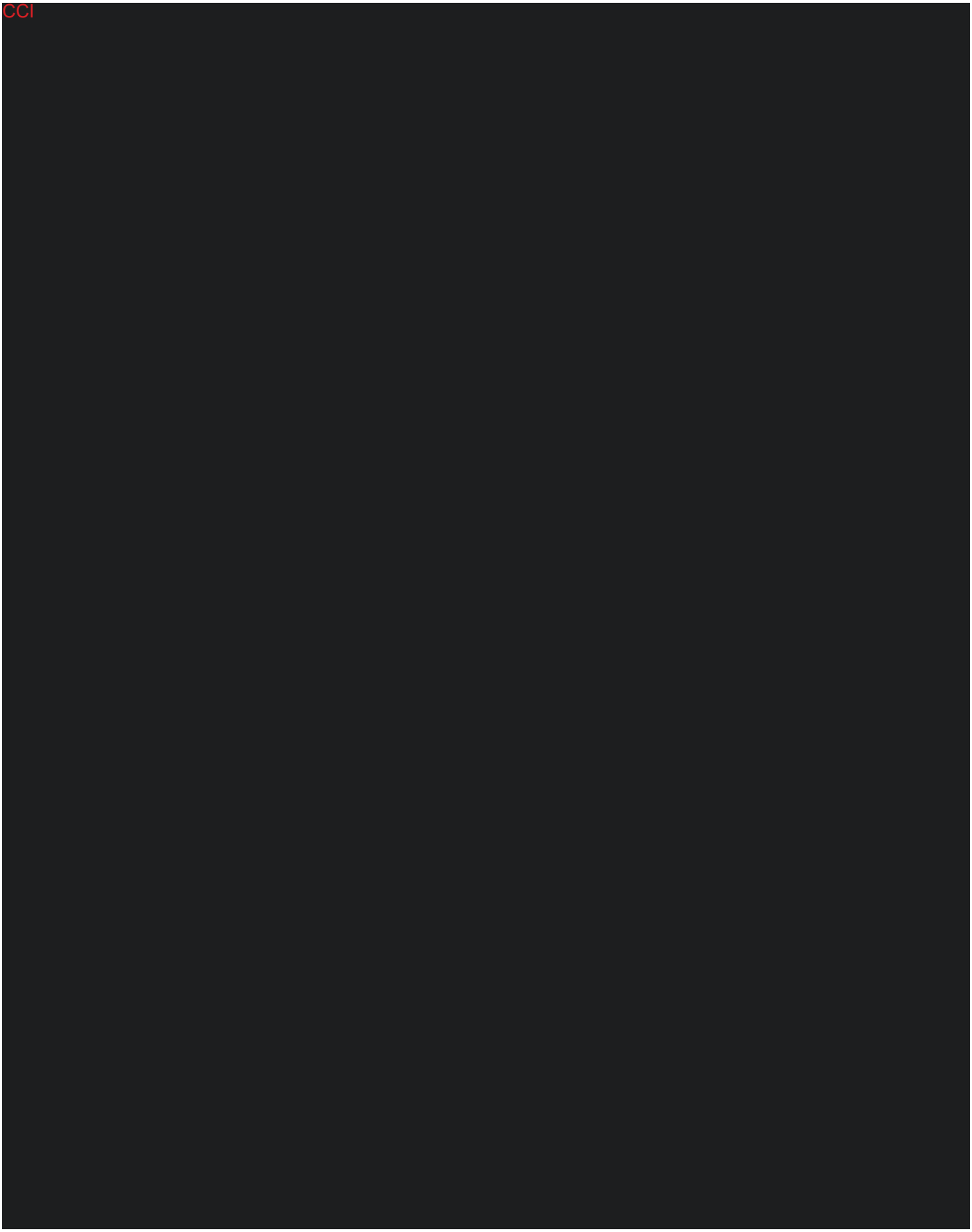
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