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**

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STATISTICAL ANALYSIS PLAN (METHODS)

PROTOCOL AG348-C-003 CORE PERIOD

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

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Protocol Number: **AG348-C-003**

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2 LIST OF ABBREVIATIONS

Abbreviation	Term
2,3-DPG	2,3-diphosphoglycerate
AE	Adverse event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATC	Anatomic Therapeutic Class
ATP	Adenosine triphosphate
BID	Twice daily
BMI	Body mass index
CI	Confidence Interval
COHb	Carboxyhemoglobin
CPAP	clinical pharmacology analysis plan
CTCAE	Common Terminology Criteria for Adverse Events
CTX	Serum C-terminal telopeptide
CTWG	Clinical Trial Working Group
DBP	Diastolic Blood Pressure
DRT	Data Review Team
DXA	Dual-energy x-ray absorptiometry
ECG	Electrocardiogram
eCRF	Electronic case report form
EPO	Erythropoietin
Hb	Hemoglobin
HCT	Hematocrit
HDL-C	High-density lipoprotein-cholesterol
Hp	Haptoglobin
HLT	MedDRA High Level Term
HR	Hemoglobin Response
LDH	Lactate dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
max	Maximum value

Abbreviation	Term
min	Minimum value
NTX	Serum N-terminal telopeptide
PD	Pharmacodynamic
PO	Administered orally
PK	Pharmacokinetic
PKD	Pyruvate kinase deficiency
PKR	Pyruvate kinase isoform R
PR	The portion of the ECG wave from the beginning of the P wave to the beginning of the QRS complex
PT	Preferred Term
q12h	Every 12 hours
QD	Once-daily
QOD	Every Other Day
QTc	Heart-rate corrected QT interval
QTcB	Corrected QT interval - Bazett correction formula
QTcF	Corrected QT interval - Fridericia's method
RBC	Red blood cell (count)
SAE	Serious adverse event
SAP	Statistical analysis plan
SBP	Systolic Blood Pressure
SD	Standard deviation
SOC	System organ class
TEAE	Treatment-emergent adverse event
TIBC	Total iron binding capacity
VS	Vital signs
WHO	World Health Organization
WHODDE	World Health Organization Drug Dictionary Enhanced

3 SUMMARY OF MODIFICATIONS

3.1 Modifications From the Approved Clinical Study Protocol

Table 3-1 Modifications from the approved clinical protocol

Protocol Section	Protocol Language	SAP Changes	Rationale
Section 12.2	Efficacy Analysis Set defined as all subjects who are enrolled and achieve at least 50% compliance with their assigned dose intensity for at least 4 weeks of continuous dosing, is specified to be the primary set for the analysis of preliminary clinical activity data.	Efficacy Analysis Set will include all subjects who received any study treatment for at least 3 weeks (Section 8.2).	A considerable number of subjects changed their actual dose from the randomized dose, usually within the first few weeks of dosing due to excessive Hb increase (a measure of efficacy). Therefore compliance as originally defined may not be relevant to efficacy measurement. In addition, the effect of PKR activators can usually be detected within a few weeks. The 3 weeks minimum treatment duration is considered sufficient to reflect the treatment effect. The Efficacy Analysis Set definition is updated to better reflect the effect of the study drug treatment on Hemoglobin response and other related clinical endpoints as detailed in Section 10.3.
Section 5.2 Section 6.2	ETCO (Core Period only) is specified as part of the 3rd bullet under the Second Objectives and subsequently considered one of the clinical activity endpoint	ETCO is removed from the objectives/endpoints	ETCO is not measured in the study as determined by the Clinical Trial Working Group (CTWG) in May 2016.
Section 12.5.3	The actual dose and duration in days of AG-348, as well as the dose intensity (computed as	Actual Dose intensity is defined as the ratio of actual dose and actual	The protocol definition for ‘dose intensity’ is the Actual dose intensity. To avoid future confusion it with planned dose intensity, the

	the ratio of actual dose received and actual duration) and the relative dose intensity (computed as the ratio of dose intensity and planned dose received/planned duration) will be listed and summarized using descriptive statistics by dose arm.	duration of study drug exposure. Relative dose intensity is defined as the ratio of actual dose intensity and planned dose intensity,	terms are updated.

3.2 Modifications From the Approved Statistical Analysis Plan

This is the first version of the final Statistical Analysis Plan for Core Period.

4 INTRODUCTION

This SAP describes the planned final analyses for the Study AG348-C-003 Core Period data and is based on the

- approved clinical study protocol (Version 5.0, dated 30 March 2016),
- approved electronic case report form (eCRF) (Version 4.0, dated 28 June, 2016),
- eCRF Completion Guidelines (Version 8.0, dated 11 August 2016).

Study AG348-C-003 is a Phase 2, open label, randomized, dose ranging study in subjects aged 18 years or older with pyruvate kinase deficiency. This study is designed to evaluate the safety, efficacy, pharmacokinetics (PK) and pharmacodynamics (PD) of AG-348.

Study AG-348-C-003 is divided into a Core Period and an Extension Period as detailed in the Protocol and summarized in Section 7.1 of this document. This SAP (Methods) documents the planned final statistical analyses of safety and efficacy analysis in the Core Period introduced in the study protocol, and describes the corresponding data presentations. It also documents additional safety and efficacy analyses not specified in the protocol, which will provide supportive information to the scientific understanding of the drug entity in the Core Period. Data from the Extension Period that becomes available at the Core Period database lock will also be included and summarized as appropriate.

The study will also evaluate the pharmacokinetic (PK) and pharmacodynamic (PD) characteristics of AG-348 in this patient population. PK and PD analyses will be documented separately in the clinical pharmacology analysis plan (CPAP) for the study.

The Agios Biostatistics and Statistical Programming (or delegates) will perform the statistical analysis of the efficacy and safety data; SAS (Version 9.2 or higher) will be used to generate all statistical outputs (tables, figures, listings, and datasets). The SAP will be finalized and approved prior to clinical data lock for the Core Period.

5 STUDY OBJECTIVES (CORE PERIOD ONLY)

5.1 Primary Objective

To evaluate the safety and tolerability of up to 24 weeks of AG-348 administration in subjects with PKD.

5.2 Secondary Objectives

- Evaluate the PK of AG-348 and the metabolite AGI-8702 (to be analyzed per CPAP).
- Evaluate the PD response of ATP and 2,3-DPG after administration of AG-348 (to be analyzed per CPAP).
- Evaluate indicators of clinical activity of AG-348 in subjects with PKD, including changes in Hemoglobin (Hb), hematocrit (HCT), reticulocyte count, haptoglobin (Hp), carboxyhemoglobin (COHb), lactate dehydrogenase (LDH), total and indirect bilirubin,

erythropoietin (EPO), hepcidin, ferritin, and transferrin saturation (serum iron/iron binding capacity).

[redacted]

6 STUDY ENDPOINTS

6.1 Safety Endpoints

- Monitoring of AEs, including determination of SAEs and AEs leading to discontinuation; safety laboratory parameters (hematology, chemistry, urinalysis, coagulation); physical examination findings; vital signs (VS); 12 lead electrocardiograms (ECGs); and DXA scans. Adverse events will be graded using CTCAE, Version 4.03. Serum sex hormone levels (testosterone [total and free], estrone, and estradiol), bone turnover markers (serum osteocalcin-N-mid and serum C-terminal telopeptide [CTX], N-terminal telopeptide [NTX]), 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.

6.2 Clinical 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.

6.2.1 Additional Endpoints

Note that the endpoints included in this section are not specified as endpoints in protocol. They are analyzed to provide additional clinical information related to Hb:

- Hb response (HR) rate in the Core Period (detailed in Section 10.3.1)
- Time to the first Hb change >=1.5 g/dL among subjects with HR in the Core Period.
- Maximum Hb change from baseline in the Core Period
- Average Hb change from baseline in the Core Period

7 STUDY DESIGN

7.1 Overall Design

Study AG348-C-003 is a Phase 2, first-in-patient, open label, two arm, multicenter, randomized, dose-ranging study in adult subjects with Pyruvate Kinase Deficiency (PKD); the study is divided into a Core Period and an Extension Period. During the Core Period, subjects receive AG-348 for up to 24 weeks; subjects who are eligible can enter the Extension Period to receive AG-348 for up to 2 years following the end of the Core Period. Subjects with PKD confirmed by red blood cell (RBC) pyruvate kinase enzymatic assay performed at Screening will be eligible to participate in this study. At Week 25 (Week 24 visit scheduled at Day 169), 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 assessments 4 weeks after the last dose of study drug. If a subject discontinues at any other time (including early discontinuation or discontinuation during the Core or Extension Period), the follow-up assessments will be conducted 4 weeks after discontinuation. 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.

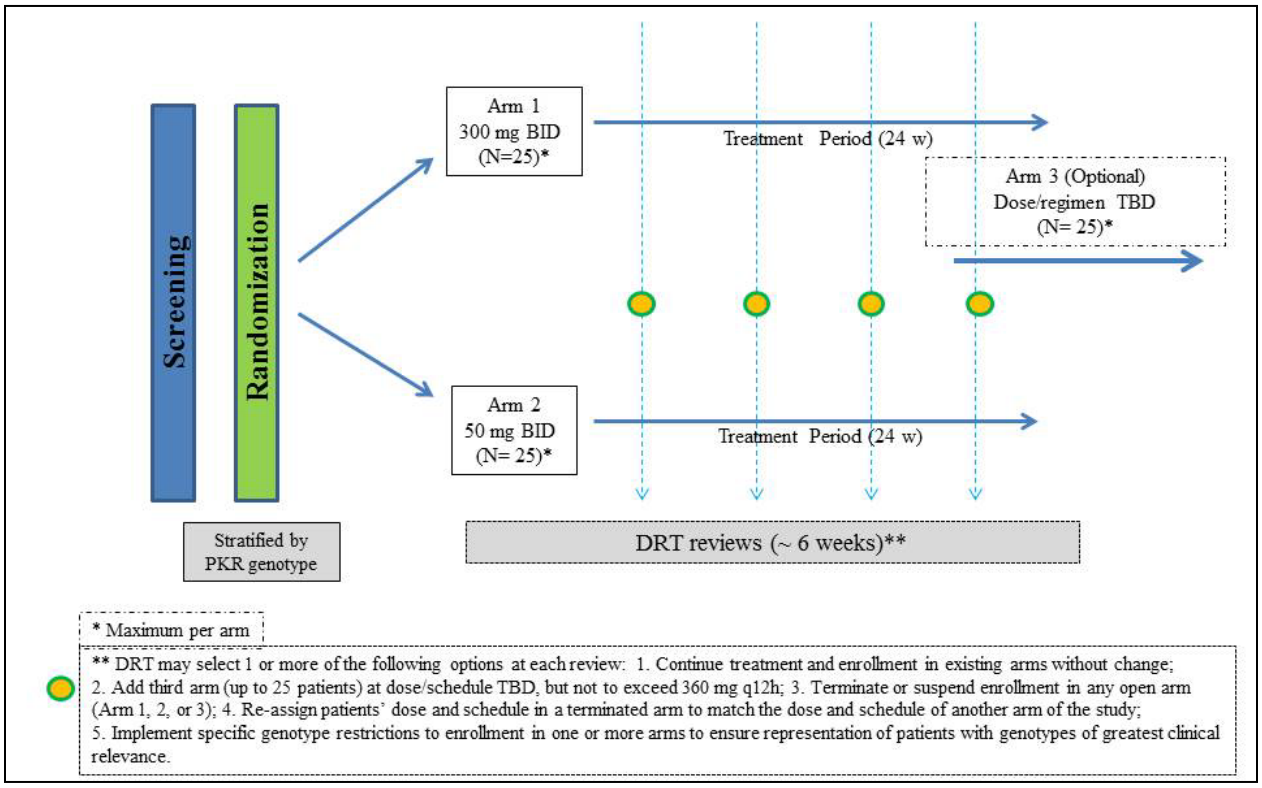
For the Core Period, approximately 25 subjects will be initially randomized on an open-label 1:1 basis to each of two twice-daily (BID) doses of AG-348 (approximately 50 subjects total, see Figure 7-1). The dose of Arm 1 is 300 mg of AG-348 administered orally (PO) every 12 hours (q12h, BID). The dose of Arm 2 is 50 mg of AG-348 administered PO q12h (BID). Randomization will be stratified by PKR mutation in order to maintain balance as much as possible across the dose arms for the specific mutations expected to be most frequently enrolled. The PKR mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H) and all other mutations (“other”). Mutation status is defined by the presence of at least one of the indicated mutations; subjects with more than one stratified mutation will be assigned based on Sponsor’s discretion.

Per study protocol, during the conduct of the study, a Data Review Team (DRT) has been established to review study data on a regular basis and adapt the study design, dose and schedule of AG-348. Based on the DRT’s recurring reviews, the DRT may 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 q12h, as this was the highest dose that demonstrated acceptable safety and tolerance in the 14-day multiple BID dosing study in healthy volunteers.

As detailed in Section 11.1, 12 DRT meetings were held during the conduct of the study before the Core Period database lock. The study remains as a 2-arm study because the DRT hasn’t exercised the option to add a 3rd arm. However, multiple patients have had their randomized dose changed, as allowed by the protocol. In some, the dose was decreased for adverse events or when the Hb exceeded the midpoint of the normal range. In some subjects initially randomized to the 50 mg BID arm, the dose was increased to 300 mg BID after at least 12 weeks of treatment if there was no Hb improvement noted with the initial lower

dose. As a result, subjects in the trial are being treated across a range of doses in the Core Period, rather than falling exclusively into the two originally assigned dose groups of 50 mg BID and 300 mg BID (Initial Dose Groups). Additional analyses were planned to fully evaluate the effect of treatment on both efficacy and safety as detailed in Section 10.

Figure 7-1 Study Schema for 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.

7.2 Sample Size and Power

Due to the rare disease setting, the minimal sample size in each dose arm may be determined by feasibility. In order to evaluate the primary objective of safety and tolerability of AG-348 in Arm 1 and Arm 2, up to a maximum of 25 patients may be randomized onto each arm. The actual number of patients enrolled into Arms 1 and 2 will depend on the safety reviews and decisions made by the DRT. In addition, up to 25 additional patients may be enrolled to evaluate an additional dose arm (Arm 3; see Section 7.1). As for Arms 1 and 2, the actual enrollment in a potential Arm 3 will depend on the safety reviews and decisions made by the DRT. Therefore, up to approximately 75 total patients may be enrolled in this study across 2 to 3 dose arms.

Table 4 provides the probability within a dose arm of detecting 1 or more AEs with varying sample size and the true underlying AE rates.

Table 7-1 Sample Size Estimation

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

7.3 Randomization

According to the initial study design, approximately 50 subjects (25 /treatment arm), who meet the eligibility criteria, will be randomized (1:1) to 1 of the 2 treatment arms, stratified by PKR mutation in order to maintain balance as much as possible across the dose arms for the specific mutations expected to be most frequently enrolled. The PKR mutation stratification factor will consist of 4 levels (R510Q, R486W, and R479H) and all other mutations ("other"). Mutation status is defined by the presence of at least one of the indicated mutations; subjects with more than one stratified mutation will be assigned based on Sponsor's discretion.

Based on the DRT's recurring reviews, the DRT may 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 q12h.

7.4 Blinding and Unblinding

Not applicable. This is an open-label study.

8 ANALYSIS SETS

The following analysis sets will be defined: Safety Analysis Set and Efficacy Analysis Set

All Subjects will be referenced to include all subjects who were randomized or dosed. Listings will be referenced using All Subjects, unless otherwise specified.

8.1 Safety Analysis Set

Safety Analysis Set will include all subjects who received at least one dose of study drug. The Safety Analysis Set will be used for safety analysis, unless specified otherwise. The primary safety analyses will be by Initial Treatment Group as in Table 8-1.

Table 8-1 Initial Treatment Group Assignments

Initial Treatment Group	Assignment
300 mg BID	Subjects who were randomized to 300 mg BID and received the dose as least once; and subjects randomized to 50 mg BID but who never received the dose and first received 300 mg BID dose
50 mg BID	Subjects randomized to 50 mg BID and received the dose as least once; and subjects randomized to 300 mg BID, never received the dose and first received 50 mg BID dose

The study allowed for dose modifications as described in Section 7.1, which leads to a range of doses that subjects received. To better reflect the analysis with appropriate treatment assignment, additional analyses will be conducted by Actual Dose Group, defined as the treatment (total daily dose) the subject received for the longest duration in the Core Period, as shown in Table 8-2.

Table 8-2 Actual Dose Group Assignment

Actual Dose Group*	Assignment
<25 mg BID	Subjects who received less than 50 mg daily dose, including 5 mg QD, 25 mg QD or 25 mg QOD for the longest duration during the Core Period
25 mg BID	Subjects who received 25 mg BID for the longest duration during the Core Period
50 mg BID	Subjects who received 50 mg BID for the longest duration during the Core Period
100 mg BID	Subjects who received 100 mg BID for the longest duration during the Core Period
200 mg BID	Subjects who received 200 mg BID for the longest duration during the Core Period
300 mg BID	Subjects who received 300 mg BID for the longest duration during the Core Period

* Additional treatment arms may appear as subjects change their treatment during the Core Period, and will be allocated accordingly.

8.2 Efficacy Analysis Set

Efficacy Analysis Set will include all subjects who enrolled and received any study treatment for at least 3 weeks. The Efficacy Analysis Set will be the set used for efficacy analysis, unless specified otherwise. The treatment assignment for Efficacy Analysis Set will primarily be by Randomized Treatment Group, as shown in **Table 8-3**.

Table 8-3 Randomized Treatment Group Assignment

Randomized Treatment Group	Assignment
300 mg BID	Subjects randomized to 300 mg BID
50 mg BID	Subjects randomized to 50 mg BID

Additional analysis will also be conducted based by Actual Dose Group as shown in **Table 8-2**.

9 ANALYSIS PERIODS

Three dosing periods, including the Core Dosing Period, Extension Dosing Period, and Cumulative Dosing Period will be defined. For each dosing period, the safety analysis will be based on their treatment-emergent period as defined below; and the efficacy analysis will be based on the corresponding efficacy window.

The **Core Dosing Period** is from the first dose date to the last dose date in the Core Period; for subjects who continued treatment in Extension Period, the last dose date in the Core Period will be imputed using the actual Week 24 visit date -1.

- The Safety Analysis will be based on treatment-emergent period in the Core Dosing Period, defined as the period from the date of the first dose in the Core Period to 1) prior to the first dose in the Extension Period for subjects who continued being dosed in the Extension Period; or to 2) the safety follow-up (if available), 30 days post last dose in the Core Dosing Period, whichever is earlier for the subjects who did not enter Extension Period.
- The Efficacy Analysis will be based on the efficacy window which is from the date of the first dose to 1) prior to first dose in the Extension Period for subjects who continued being dosed in the Extension Period; or to 2) 1 day after the end of core dosing period (to account for the assessment collected the next day after the last dose) for subjects who did not dose in Extension Period.

The Extension Dosing Period is from actual Week 24 visit date to the last dose date in the Extension Period for subjects who continued treatment in Extension Period.

- The Safety Analysis will be based on the Treatment-emergent period in Extension Dosing Period, which is from the date of the first dose in the Extension Period to 1) the safety follow-up (if available) or 30 days post last dose whichever is earlier for the subjects who

completed or discontinued treatment in Extension Period; or 2) to the date of Core Period database lock for subjects who are still on treatment in the Extension Period.

- The Efficacy Analysis will be based on the efficacy window which is from the first dose in the Extension to 1 day after the last dose in the Extension Dosing Period.

The Cumulative Dosing Period is from the date of the first dose (in the Core Period) to the last dose date (in the Core period for subjects who did not dose in Extension Period and in the Extension Period for subjects who dosed in Extension Period). For subjects who are still on treatment in the extension period, the date of Core Period database lock will be used as the subject's last dose date.

- The Safety Analysis will be based on the Treatment-emergent period in Cumulative Period, defined as from the first dose date to the safety follow-up visit (if available) or 30 days post last dose date whichever is earlier.
- The Efficacy Analysis will be based on the efficacy window which is from the first dose date to 1 day (inclusive) after last dose in the Cumulative Dosing Period.

10 STATISTICAL ANALYSIS

10.1 General Considerations

The Schedule of Assessments is provided in Appendix A.

Continuous variables will be summarized using the following descriptive summary statistics: the number of subjects (n), mean, standard deviation (SD), median, minimum value (min), and maximum value (max).

The precision of the measurement in raw data for other continuous variables will be used to determine the number of decimal places to present in tables, figures, and derived listings. Minimum and maximum values will be reported with the same precision as the units of measure. The mean and median will be reported to 1 greater decimal place. SD will be reported to 2 greater decimal place.

Categorical variables will be summarized using counts and percentages. Percentages will be presented to 1 decimal place.

Baseline Value: Unless otherwise specified, the baseline value will be defined as the most recent non-missing measurement (scheduled or unscheduled) collected before the initial administration of study drug. Unscheduled visit measurements will be considered for derivation of baseline.

- The baseline of Hb will be calculated as the arithmetic average of all available central lab results within 6 weeks (42 days) prior to the first dose, excluding Hb within 2 months (61days) of a transfusion.

Change (Absolute Change) from baseline: will be calculated as Postbaseline value - Baseline value.

Relative change from baseline: will be calculated and expressed in percentages as 100×(Postbaseline value - Baseline value)/Baseline value.

Analysis visits: Nominal visits will be used in the by-visit summaries. If there are multiple assessments collected with the same nominal visit, the assessment closest to the targeted visit date will be used. The actual study day will be included in listings.

Unscheduled Visits: Unscheduled visit measurements will be included in listings and for the analysis of max/min values and max/min changes from baseline values.

Central and Local Lab: Only central lab data will be used for by-visit summary including the actual values and their changes from baseline. Local lab data, in addition to central lab data, will be included in shift tables and the summary tables of categorized worst value or change for safety analysis. Both central and local labs will be included in the listings.

Repeated Measurements: For repeated measurements, i.e., measurements with exactly the same collection date and time (if time is available), the average of the repeated measurements will be used to reflect the value at the corresponding measurement date and time. Actual values will be presented in the data listings.

Incomplete/Missing data will not be imputed, unless otherwise specified; i.e., all missing values will remain as missing in all statistical analyses and listings, unless otherwise specified.

Outliers: No formal statistical analyses will be performed to detect and/or remedy the presence of statistical outliers.

10.2 Background Characteristics

10.2.1 Subject Disposition

The number and percentage of subjects in the following analyses sets will be summarized:

- Randomized (number only)
- Treated (Number, with percentage calculated based on the number of subjects randomized)
- Efficacy Analysis Set
- Safety Analysis Set

The percentage will be calculated based on the number of treated subjects.

The number and percentage of subjects in each disposition category below will be summarized with the number in the Safety Analysis Set as the denominator:

- Completed Core Period treatment
    - Extension enrollment status (Yes vs. No)
- Prematurely discontinued treatment in Core Period
    - Reasons for discontinuations

Among subjects who enrolled into Extension, the number and percentage of subjects in the following category will be provided, with the number of Safety Analysis Set as the denominator:

- On Treatment (at the time of Core Period Database Lock)
- Completed Extension Period treatment
- Prematurely discontinued treatment in Extension Period
    - Reasons for discontinuations

Of the subjects who completed or discontinued study, the reason they are off study.

The disposition table will be provided by Initial Treatment Group and repeated by the Actual Dose Group.

A listing will be provided including 1) subjects who discontinued treatment in the Core Period; 2) subjects who completed treatment in the Core Period but did not roll into the Extension Period; 3) and subjects who discontinued treatment in the Extension Period.

10.2.2 Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized based on the Safety Analysis Set by the Initial Treatment Group, and repeated by the Actual Dose Group:

- Age at informed consent (in years)
- Sex (female and male)
    - Female subjects will further be summarized by their child bearing potential status (Yes, No, and missing), with the percentage calculated using number of female subjects;
    - Female subjects with child bearing potential will be summarized by their usage of adequate birth control (Yes, No, and missing), with the percentage calculated using number of female subjects with child bearing potentail;
- Race (White, Black or African American, Asian, American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, Not Reported, Other [including subjects reported with one race])
- Ethnicity (Hispanic or Latino, not Hispanic or Latino, and Not Reported)

  For subjects who reported multiple races, they will be considered as ‘Other’.
- Baseline characteristics include
    - Weight, height and body mass index (BMI)
    - Hemoglobin level
    - DXA Scan by location (Hip, Spine, and Femoral Neck): Bone mineral density and their corresponding T-scores and Z-scores

- PKR genotype related
  - PKR stratification factors (R510Q, R486W, R479H, and Others),
  - PKR mutations for each allele
  - PKR mutation groups (missense/missense; missense/non-missense; non-missense/non-missense)
- Disease characteristics include
  - Age at PKD diagnosis
  - Splenectomy history (Yes or No): Of those with splenectomy, their age at splenectomy, type (total or partial) and effect on transfusion burden (reduced or not) and baseline hemoglobin (increased or not),
  - Cholecystectomy history (Yes vs. No), age at cholecystectomy among those with splenectomy,
  - Chelation history (Yes or No),
  - Phlebotomy during the 12 months prior to enrollment

Demographics, baseline characteristics and disease characteristics will be included in subject level listings.

Targeted medical history by reported term and Medical history by System organ class (SOC) and Preferred Term (PT) summary will be presented for the Safety Analysis Set.

10.2.3 Prior and Concomitant Medications

Medications used in this study will be coded by using the World Health Organization Drug Dictionary Enhanced (WHODDE) and categorized as the following:

- **Prior medication:** any medication that started before the first dose date of study drug of the Core Dosing Period, regardless of when the medication ended.
- **Concomitant medication:** medication continued or newly received on or after the first dose date of study drug through the end of treatment-emergent period.

  Concomitant medication will be summarized for the Core Dosing Period and repeated for the Cumulative Dosing Period.
- **Post-treatment medication:** medication continued or newly received after the treatment-emergent period.

A given medication can be classified as a prior medication, a concomitant medication, or a post-treatment medication; both prior and concomitant; both concomitant and post-treatment; or prior, concomitant, and post-treatment. If a medication has a missing or partially missing start/end date or time and it cannot be determined whether it was taken before initial dosing, concomitantly, or more than 30 days after the last dose of study drug, it will be considered as prior, concomitant, and post-treatment.

Prior medications, concomitant medications for the Core Dosing Period and concomitant medications for the Cumulative Dosing Period will be summarized descriptively by anatomic class (ATC) level 1, ATC level 2, and preferred name based on the Safety Analysis Set. Post-treatment medication will only be included in the subject level listings.

10.2.4 Study Drug Exposure

Exposure will be summarized for Core Dosing Period and the Cumulative Dosing Period based on the Safety Analysis Set by Initial Treatment Group.

Duration of study drug exposure is defined as: last dose date – first dose date + 1 day, regardless of any interruptions in dosing. Duration of study drug exposure will be summarized descriptively (n, mean, SD, median, min, and max) and in categories (>0 to ≤2 weeks, >2 to ≤4 weeks, >4 to ≤8 weeks, >8 to ≤16 weeks, >16 to ≤24 weeks, >24 to ≤52 weeks, >52 to ≤108 weeks and >108 weeks).

10.2.5 Study Drug Compliance

Compliance will be summarized for both the Core Dosing Period and the Cumulative Dosing Period based on the Safety Analysis Set by Initial Treatment Group. Given the lag in data entry of study drug compliance in the Extension period at the time of Core Period database lock, compliance during the Cumulative Dosing Period is only based on data reported and needs to be interpreted with caution.

Actual dose is the total amount of study drug taken (mg), calculated as the sum of the amount of drug taken for all dosing regimens while the subject is in the corresponding dosing period. The doses for each dosing regimen is the duration of the dosing regimen times the dose times the frequency of the dose. Note that by this derivation, the missing exposure records during the core period is considered as no study drug taken during the corresponding interval.

Planned dose is the planned total amount of study drug (mg), calculated as the sum of the planned drug amount expected to be taken for each interval in the corresponding dosing period. The planned drug amount expected to be taken for each interval is calculated as the duration of treatment times the dose times the dosing frequency for the exposure reported as ‘Per Protocol’, ‘Dose Increased’, ‘Dose Reduced’ or ‘Dose Held’. For the records reported as ‘Patient Missed Dose(s)’ or ‘Incorrect Dose’ or missing reason for dose change, the dose level and frequency from the last available compliance records prior will be used.

Compliance will calculated as the ratio of actual dose and the planned dose.

Actual dose, planned dose, and compliance will be summarized descriptively (n, mean, SD, median, min, and max). The number and percentage of subjects with compliance in different categories (i.e., <80%, ≥80% - <90%, ≥90% - <95%, vs ≥95%) will be summarized.

10.2.6 Actual Dose Intensity and Relative Dose Intensity

Actual Dose intensity is defined as the ratio of actual dose and actual duration of study drug exposure. Relative dose intensity is defined as the ratio of actual dose intensity and planned dose intensity, i.e., daily dose for randomized treatment (100 mg for those randomized to 50

mg BID group and 600 mg for those randomized to 300 mg BID group). Both dose intensity and relative dose intensity will be summarized for the Core Dosing Period and the Cumulative Dosing Period based on the Safety Analysis Set by Initial Treatment Group.

10.2.7 Study Drug Modification

Number and percentage of subjects with dose held or reduced, Number and percentage of subjects with dose increased will be summarized respectively based on the Safety Analysis Set by the Initial Treatment Group and repeated by the Actual Dose Group. The subjects with dose held or reduced will further be summarized by reason (Adverse event, increased hemoglobin, and others).

10.2.8 Protocol Deviations

All protocol deviations/violations as reported by the site monitor will be provided as a subject data listing.

10.3 Efficacy Analysis

Efficacy summary tables listed below will be summarized based on the Efficacy Analysis Set overall, and by Randomized Treatment Group. The summary will also be repeated by the Actual Dose Group, unless specified otherwise.

10.3.1 Analyses of Hemoglobin (Hb)

Hb values and their changes from baseline will be summarized at scheduled visits (including visits from the Core Period and the visits from the Extension Period).

Unless specified otherwise, only central lab results will be considered in the Hb related analyses. Hb results obtained within 2 months (61 days) after a red blood cell transfusion will be excluded.

Hemoglobin response

A hemoglobin response (HR) is defined as having post-baseline changes in Hb ≥1.5 g/dL at >50% assessments in the Core Dosing Period Efficacy Window. The Number and percentage of subjects with a HR will be summarized, along with the 95% CI based on exact binomial distribution.

Mean (95% Confidence Interval [CI]) of Hb values at scheduled visits will be plotted by HR status (as defined below). Spaghetti plot for Hb assessments (excluding the Hb within 2 months of transfusion) and spaghetti plot of Hb change from baseline will be provided by HR status. In spaghetti plots, both central and local lab will be included. In cases where there are both central and local labs on the same date, the central lab result will be used.

The time to the first occurrence of Hb change from baseline >1 g/dL, ≥1.5 g/dL and ≥2 g/dL for subjects with a HR will be summarized using summary statistics.

Maximum Hb change

The Maximum Hb change from baseline will be summarized using descriptive statistics. In addition, categorical analysis (number and percentage of subjects) of maximum Hb change

(g/dL) from baseline (<1, 1 - <1.5, 1.5 - < 2, ≥2) will be summarized. A waterfall plot of maximum Hb change from baseline will be provided. These analyses will be repeated by HR status (Yes vs. No).

The maximum Hb change from baseline considering only assessments collected while the subject was on the treatment same as the Actual Dose Group for at least 2 weeks will also be summarized for Hemoglobin responders.

Average Hb change

Average Hb change from baseline, defined as the arithmetic average of all Hb changes from baseline at all scheduled post-baseline visits based on central lab, excluding those obtained within 2 months (61 days) after a red blood cell transfusion, will be summarized using descriptive statistics. In addition, categorical analysis (number and percentage of subjects) of average Hb change (g/dL) from baseline (<1, 1 - <1.5, 1.5 - < 2, ≥2) will be summarized. Waterfall plot of average Hb change from baseline will be provided. These analyses will be repeated by HR status (Yes vs. No).

The analyses for maximum Hb change and average Hb change will be repeated based on Efficacy Analysis Set by Actual Dose Group.

The Hb by-visit summary, analysis of maximum Hb change and average Hb change, Hemoglobin response (Yes. vs. No) will be repeated by PKR genotype (Missense/Missense, Missense/Non-Missense, Non-Missense/Non-Missense) based on Efficacy Analysis Set by Actual Dose Group.

All available Hb values, including central and local labs, for all subjects will be listed.

10.3.2 Other Preliminary Clinical Activity Indicators

Absolute values of HCT, percentage and absolute reticulocyte count, Hp, COHb, LDH, total and indirect bilirubin, EPO, and their changes from baseline will be summarized at each scheduled visit by HR status using summary statistics. Mean (±SD) by visit will be plotted by HR status.

For the summary of iron related parameters, including hepcidin, ferritin, iron, TIBC, and transferrin saturation, the summary will be separated for subjects reported on chelation over the efficacy analysis window vs. those reported no chelation. For subjects reported a change in chelation status during the efficacy analysis window, their related assessments will not be included in the summary table. Note that there is a testing method/kit change in hepcidin in the middle of the trial and the related results need to be interpreted with caution.

These assessments will also be listed.

10.4 Safety Analysis

The overall safety profile of study drug will be assessed in terms of the following safety and tolerability endpoints:

- Adverse events

- Clinical laboratory values (hematology, serum chemistry, coagulation studies, and urinalysis)
- Assessment of Aromatase Inhibitor Activity:
    - DXA scans
    - Serum sex hormones (testosterone [total and free], estrone, and estradiol)
    - Bone turnover markers (serum osteocalcin-N-mid and serum C-terminal telopeptide [CTX], N-terminal telopeptide [NTX]), 25-hydroxy vitamin D2 and D3, total cholesterol, high-density lipoprotein-cholesterol (HDL-C)
- Standard digital electrocardiograms (ECGs)
- Vital signs
- Physical exams

Safety endpoints will be analyzed based on the Safety Analysis Set by Initial Treatment Group and repeated by the Actual Dose Group, unless specified otherwise. The listings will be provided for the Cumulative Dosing Period. Only descriptive analysis of safety will be performed (i.e., no statistical testing will be performed).

10.4.1 Adverse Events

For analysis purpose, AEs will be categorized as pretreatment AEs, treatment-emergent adverse events (TEAEs), or post-treatment AEs:

- **Pretreatment AE:** any AE that started in the pre-treatment period.
- **TEAE:** any AE that increased in severity or that was newly developed in the treatment-emergent period.
- **Post-treatment AE:** any AE that increased in severity or that was newly developed after the end of treatment-emergent period.

For AEs with missing or partial start dates, if there is no clear evidence that the AEs started before or after study treatment, then the AEs will be classified as TEAEs.

Treatment-emergent AEs (TEAEs) for the Core Period and the Cumulative Period will be summarized, separately, based on Safety Analysis Set by Initial Treatment Group. Additional analyses based on the Safety Analysis Set by Actual Dose Group will also be provided.

An overview of the TEAE profile will be provided, including number and percent of subjects for the following categories:

- All TEAEs
- TEAEs by relationship
- TEAEs by maximum severity
- TEAEs leading to treatment modification
- TEAEs leading to treatment interruption

- TEAEs leading to treatment discontinuation
- Serious TEAEs
- Related serious TEAEs
- TEAE leading to death

Adverse events summary tables will be presented for TEAEs only and will include the following:

- All TEAEs
- Grade 3/4 TEAEs
- Related TEAEs (including Possibly Related and Probably Related)
- TEAEs by relationship
- TEAEs leading to treatment modification
- TEAEs leading to treatment interruption
- TEAEs leading to treatment discontinuation
- Serious TEAEs
- Related serious TEAEs
- TEAEs leading to death
- TEAEs by treatment interval for the Core Dosing Period (>0 to ≤1 Week: [Day1, Day8], >1 to ≤2 Weeks: [Day9, Day15], >2 to ≤8 Weeks: [Day16, Day57], >0 to ≤8 Weeks: [Day1, Day57], >8 to ≤16 Weeks: [Day58, Day113], >16 to ≤24 Weeks: [Day114, Day169], >24 Weeks: [Day170, end of TEAE period])

Summaries will be presented by Medical Dictionary for Regulatory Activities (MedDRA) system organ class (SOC) and preferred term (PT) using frequency counts and percentages (i.e., number and percentage of subjects with an event). When summarizing the number and percentages of subjects, subjects with multiple occurrences of the same adverse event or a continuing adverse event will be counted once, and only the maximum severity level will be presented in the severity summaries, and the worst/highest relationship level in the relationship summaries.

In addition to the summary of all TEAEs by SOC and PT, TEAEs in the Core Period by PT will also be provided based on the Safety Analysis Set by Initial Treatment Group.

Additional Events of interest:

AEs of endocrinological interest, defined by the AEs whose High level terms (HLTs) or PTs as listed in Appendix F will be summarized by total number of subjects with menopause symptoms, as well as by corresponding SOCs and PTs using frequency counts and percentages.

A listing of AEs will be provided. In addition, listings containing individual subject adverse event data for TEAEs leading to treatment interruption/interruption/discontinuation, SAEs and all deaths will be provided separately. In the listing of SAEs and death, a flag indicating the TEAE status will be included.

10.4.2 Clinical Laboratory

For the laboratory measurements, the raw values and change from baseline values of the continuous hematology, chemistry, special chemistry, coagulation and urinalysis results will be summarized at each scheduled time point. For parameters summarized in Table 12-1, the original reporting units will be used. All other parameters will be summarized in SI units.

Listings containing individual subject hematology, chemistry, and coagulation values outside the reference ranges will be provided. This listing will include data from scheduled and unscheduled time points.

Analysis on liver function test (LFT)

- For transaminase (AST and ALT),
    1) Box plot of LFT value/ULN will be plotted against visit.
    2) Shift of LFTs by CTCAE grade from baseline to the worst grade during the treatment-emergent period will also be summarized for AST and ALT separately. In addition, subjects with AST shift or ALT shift will be summarized. In this AST or ALT pooled summary, the shift of the parameter with the worst grade in the treatment-emergent period is considered; when AST and ALT have the same worst grade in the treatment-emergent period, the shift of the parameter with the lowest baseline grade is considered.
    3) A listing of subjects with elevated AST and ALT results during the treatment-emergent period will be presented. The listing will include both AST and ALT at all visits.
- For all LFT results (alanine aminotransferase [ALT], aspartate aminotransferase [AST], and serum alkaline phosphatase [ALP]), a spaghetti plot (including all time points) will be provided under the unit of ULN:
    1) The plot for ALT will include subjects with at least one ALT>3×ULN during the treatment-emergent period.
    2) The plot for AST will include subjects with at least one AST>3×ULN during the treatment-emergent period,
    3) The plot for ALP will include subjects with at least one ALP>1.5×ULN during the treatment-emergent period, and

Analysis on triglycerides and cholesterol

Shift of triglycerides and cholesterol by CTCAE grade from baseline to the worst grade during the treatment-emergent period will be summarized. Mean values (±SD) against visit will be provided for triglycerides, cholesterol and HDL-C. A listing of subjects with elevated

triglycerides during the treatment-emergent period will be presented. The listing will include triglycerides, cholesterol and HDL-C at all visits.

10.4.3 Standard Digital ECG

For the ECG measurements during the treatment-emergent period, a summary of raw values and change from baseline values will be provided at each scheduled time point for the following standard digital ECG measurements: PR, QT, and QT corrected for HR (QTc) intervals (Fridericia's correction [$QTcF = QT/RR^{1/3}$], QRS duration, and HR.

In addition, number and percentage of subjects with QTcF in categories of ≤450, >450-≤480, >480-≤500 and >500 and the changes in QTcF from baseline in categories of ≤0, >0-≤30, >30-≤60, and >60 will be provided.

10.4.4 Vital Signs

For the vital signs measurements, the raw values and change from baseline values will be summarized at each scheduled time point: weight, BMI (calculated as weight [kg]/height $[m]^2$), with height using the last available height), systolic and diastolic blood pressure (mm Hg), body temperature (°C), HR (beats per minute), and respiratory rate (breaths per minute).

To further evaluate the impact of triglycerides on weight, weight and their change from baseline will be summarized by visit separating by subjects who had at least 1 grade shift versus those remain at the same grade in triglycerides. This subgroup analysis will be done only by Actual Dose Group based on the Safety Analysis Set.

10.4.5 Physical Examination

PE findings will be presented as a data listing only.

10.4.6 Assessment of Aromatase Inhibitor Activity of AG348

- Sex Hormones: testosterone (total and free), estrone, and estradiol.

  For sex hormones, the by-visit summary will be provided by sex based on the Safety Analysis Set. A spaghetti plot will also be provided by Actual Dose Group and by sex. The spaghetti plot will be repeated to only include assessments within the efficacy analysis window of the Cumulative Dosing Period to present the subjects' on-treatment hormone profile. Note that there is a testing method/kit change in free testosterone in the middle of the trial and the related results need to be interpreted with caution.
- Bone Turnover: serum osteocalcin-N-mid, CTX, and NTX: The by-visit summary will be provided by sex.
- Lipids: total cholesterol, HDL-C, triglycerides: The by-visit summary will be provided by sex.
- DXA: bone mineral density at each visit (Baseline, week 24 in core period; month 18, month 30 in extension period) will be summarized; The summary will be provided by location (hip vs. spine vs. femoral neck) and by sex.

- For bone mineral density, in addition to the actual value and their change from baseline, the percent changes from baseline will also be summarized.
- For the t-scores and z-scores, only the actual value and their change from baseline will be summarized. Additional categorical (<-2.5, -2.5-<-1, -1-<0, >=0) summary will be provided for both t-score and z-score.

All Sex Hormone, Bone Turnover, lipids, and DXA results will be listed. In the listing for Sex Hormone, the number of days of last menstrual cycle preceding the assessment (calculated as the assessment date – last menstrual cycle start date + 1) will be provided for female subjects.

10.4.7 Menstrual Cycle Diary

Menstrual cycle diary will be summarized by regular contraceptive status (oral contraceptives or depot injection) for subjects with child bearing potential. Total number of menstrual cycle will be summarized. Number and percentage of menstrual cycles that are heavier, lighter, longer, shorter, sooner and later than usual will be summarized, where the percentage will be calculated based on the total number of reported menstrual cycles.

Of the women who reported at least one menstrual cycle difference from usual, number and percentage of subjects with at least one menstrual that is heavier, lighter, longer, shorter, sooner and later than usual will be provided. The summary will be separated for female subjects with child bearing potential who ever reported using regular contraceptives (oral contraceptives or depot injection) versus those who never reported using regular contraceptives.

A listing of menstrual diary will be provided.

11 INTERIM AND DMC ANALYSES

11.1 Interim analysis/DRTs

There were 12 DRT reviews of the ongoing data. Based on the on-going DRT review, the study retained the 2 planned arms (300 mg BID and 50 mg BID as initially designed.

DRT13 will be held based on the final analysis described above.

11.2 DMC Analysis

Not DMC is conducted for this study.

12 APPENDICES

Appendix A Schedule of Assessments

Note: All the sections referenced in the footnotes refer to sections in the protocol rather than the current SAP.

Table 4-1: Schedule of Assessments: Core Period

Timing	**Pre-Treatment**		**Month 1**				**Months 2 and 3**			**Months 4, 5 and 6**			**Follow Up**[1]
Visit	**Screening**		**Baseline / D1**	**W1**	**W2**	**W3**	**W6**	**W9**	**W12**	**W16**	**W20**	**W24**	**W28**
Study Day	**-42 to -1**	**-40 to -1**[2]	**1**	**8**[3]	**15**	**22**[3]	**43**	**64**	**85**	**113**	**141**	**169**	**197**
Visit Window				**± 2 D**	**± 2 D**	**± 2 D**	**± 7 D**	**± 7 D**	**± 7 D**	**± 7 D**	**± 7 D**	**± 7 D**	**± 7 D**
Written Informed Consent	X												
PK enzyme assay (confirmation of PK deficiency)[4]	X												
PKR Genotype (for randomization)	X												
UGT1A1 Genotype	X												
Demographics	X												
Medical/Surgical History (General and PK deficiency-specific) [5]	X												
Medication History	X												
Transfusion History	X												
Confirmation of Vaccinations (Splenectomized Patients)	X												
Physical Examination[6]/ Height[6] and Weight	X		X		X			X	X	X	X	X	X

Timing	**Pre-Treatment**		**Month 1**				**Months 2 and 3**			**Months 4, 5 and 6**			**Follow Up**[1]
Visit	**Screening**		**Baseline / D1**	**W1**	**W2**	**W3**	**W6**	**W9**	**W12**	**W16**	**W20**	**W24**	**W28**
Study Day	**-42 to -1**	**-40 to -1**[2]	**1**	**8**[3]	**15**	**22**[3]	**43**	**64**	**85**	**113**	**141**	**169**	**197**
Visit Window				**± 2 D**	**± 2 D**	**± 2 D**	**± 7 D**	**± 7 D**	**± 7 D**	**± 7 D**	**± 7 D**	**± 7 D**	**± 7 D**
Performance Status	X		X		X			X	X	X	X	X	X
Vital signs (BP, HR, RR, T)[7]	X		X	X	X	X	X	X	X	X	X	X	X
12-lead ECG[8]	X		X	X		X						X	X
DXA Scan[9]	X											X[10]	
Laboratory Evaluations [11]													
HBsAg, HCV Ab, HIV1 and 2 Ab	X												
RBC antibody Screen	X												
Hematology (CBC)[12]	X	X[13]	X	X	X	X	X	X	X	X	X	X	X
Haptoglobin[14]			X			X			X			X	X
EPO levels[15]			X			X			X			X	X
G6PD screen	X												
Hepcidin			X			X			X			X	X
Serum Chemistry[16]	X		X	X	X	X	X	X	X	X	X	X	X
Iron Panel[17]			X						X			X	
Carboxyhemoglobin (COHb)[18]			X			X	X	X	X	X	X	X	
ETCO[19]	X		X	X	X	X	X	X	X	X	X	X	X

Timing	**Pre-Treatment**		**Month 1**				**Months 2 and 3**			**Months 4, 5 and 6**			**Follow Up**[1]
Visit	**Screening**		**Baseline / D1**	**W1**	**W2**	**W3**	**W6**	**W9**	**W12**	**W16**	**W20**	**W24**	**W28**
Study Day	**-42 to -1**	**-40 to -1**[2]	**1**	**8**[3]	**15**	**22**[3]	**43**	**64**	**85**	**113**	**141**	**169**	**197**
Visit Window				**± 2 D**	**± 2 D**	**± 2 D**	**± 7 D**	**± 7 D**	**± 7 D**	**± 7 D**	**± 7 D**	**± 7 D**	**± 7 D**
Coagulation Studies [20]	X		X		X				X			X	X
Urinalysis[21]	X		X		X				X			X	X
Serum or Urine Pregnancy[22]	X		X										
Lipids[23]			X				X		X			X	X
Hormonal Testing[24]	X	X[25]	X						X			X	X
Serum osteocalcin-N-mid and CTX[26]			X						X			X	
25-hydroxy Vitamin D2 and D3			X						X			X	
Randomization[27]	X												
Study Drug Administration			X	X	X	X	X	X	X	X	X	X[28]	
Dispense Study Drug[29]			X	X	X	X	X	X	X	X	X		
PK blood sampling[30]			X		X	X	X	X	X	X	X	X	
PD Assessments[30]													
2,3-DPG/ATP			X		X	X	X	X	X	X	X	X	

Timing	**Pre-Treatment**		**Month 1**				**Months 2 and 3**			**Months 4, 5 and 6**			**Follow Up**[1]
Visit	**Screening**		**Baseline / D1**	**W1**	**W2**	**W3**	**W6**	**W9**	**W12**	**W16**	**W20**	**W24**	**W28**
Study Day	**-42 to -1**	**-40 to -1**[2]	**1**	**8**[3]	**15**	**22**[3]	**43**	**64**	**85**	**113**	**141**	**169**	**197**
Visit Window				**± 2 D**	**± 2 D**	**± 2 D**	**± 7 D**	**± 7 D**	**± 7 D**	**± 7 D**	**± 7 D**	**± 7 D**	**± 7 D**
Dispense/Collect Menstrual Cycle Diary [32]			X				X		X	X	X	X	X
Adverse Events[33]			Continuous										X
Transfusion Record[34]	X		X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications/Procedures	X		X	X	X	X	X	X	X	X	X	X	X
Rollover to Extension Period[35]												X	

Abbreviations: Ab = antibody; ATP = adenosine triphosphate; BP = blood pressure; CBC= complete blood count; COHb = carboxyhemoglobin; CTX = C-terminal telopeptide; D = day; DPG = diphosphoglycerate; DXA = Dual-energy x-ray absorptiometry; ECG = electrocardiogram; EPO = erythropoietin; ETCO = end tidal carbon monoxide; FSH = follicle stimulating hormone; G6PD = glucose-6-phosphate-dehydrogenase; HBsAg = hepatitis B surface antigen; HCV Ab = hepatitis C virus antibody; HDL-C = high-density lipoprotein-cholesterol; HIV = human immunodeficiency virus; HR = heart rate; PD = pharmacodynamic; PK = pharmacokinetic; PK deficiency = pyruvate kinase deficiency; PKR = pyruvate kinase isoform R; RR = resting rate; W = week.

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

1 The Week 28 Follow-up Visit will only be conducted for patients who do not enter the Extension Period.

2 To be performed at least 2 days after the first Screening Visit.

3 In-clinic visits on Day 8 and Day 22 may be performed by the patient's primary care physician if necessary and must be approved by the Sponsor on a case by case basis; in these instances PK/PD sampling would not be required and dispensing of study medication would not be performed. For the Day 8 visit performed by the patient's primary care physician, the primary care medical office will collect a blood sample for hematology using the blood sample collection and shipping supplies from the kit prepared by the designated central laboratory. The kit will be sent to the primary care physician's office. No other testing or procedures will be asked of the primary care physician on Day 8 [VS and serum chemistry will not be required]. The 12-lead ECG scheduled for Day 8 will instead be performed at the main study center on Day 15. For the Day 22 visit performed by the patient's primary care physician, the primary care medical office will collect a blood sample for hematology using the blood sample collection and shipping supplies from the kit prepared by the designated central laboratory. The kit will be sent to the primary care physician's office. No other testing or procedures will be asked of the primary care

physician on Day 22. [VS, serum chemistry, coagulation, haptoglobin, EPO level, carboxyhemoglobin, and PK/PD samples will not be required.] The 12-lead electrocardiogram scheduled for Day 22 will instead be performed at the main study center on Day 43 (Week 6). For patients having their Day 8 and/or Day 22 visits performed by the primary care physician, the Principal Investigator will him or herself, or have a qualified research nurse or other designated site staff member, make telephone contact with the patient to inquire about any adverse events. These must be recorded as if the patient appeared in the main study center. The Principal Investigator will exercise prudent clinical judgment in determining any clinical course of action to take based on any adverse events discovered. These telephone contacts on Day 8 and Day 22 must be explained to the patient in advance and scheduled in advance to maximize the likelihood of successfully making contact.

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

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

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

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

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

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

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

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

[12] Three Screening/Baseline samples will be collected for complete blood count (CBC). Samples will be collected in the AM on 3 different days; the samples collected on Baseline/Day 1 may comprise one of these; samples may be taken at the same time as Screening/Baseline hormone assessments (any 2 time points during Screening at least 2 days apart). CBC will include HCT, Hb, red blood cell (RBC) count, absolute reticulocyte count, percent reticulocyte count, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count with automated (or manual, as indicated) differential, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and platelet count. In the event that the designated central laboratory for the study is unable to provide a valid result for any specific component of the defined CBC for a specific patient, the site may be asked to have the test performed at their local laboratory. The result of the local CBC will be entered into the study database along with the local normal reference range.

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

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

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

[16] Alkaline phosphatase, sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide (CO2) or bicarbonate, albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and indirect bilirubin, (and estimated creatinine clearance or glomerular filtration rate for screening only, as appropriate).

[17] Iron, total iron-binding capacity (TIBC), transferrin saturation, and ferritin will be performed prior to dosing on Day 1, at the end of Week 12 and at the end of Week 24.

[18] To be collected before the AG-348 morning dose is administered.

[19] End tidal carbon monoxide (ETCO) assessment by CoSense® End Tidal Carbon Monoxide Monitor to be performed only at a subset of investigative sites that elect to participate in this assessment and only during the Core Period. The screening ETCO may be performed at either Screening 1 or Screening 2. ETCO measurement should performed before administration of AG-348 on Baseline/Day 1 and on W2, W3, W6, W9 W12, W16, W20, W24, and W28 (W28 only if not going into Extension Period).

[20] Fibrinogen, activated partial thromboplastin time (aPTT), and international normalized ratio (INR) will be performed at Screening, prior to dosing on Day 1, at the end of Week 2, the end of Week 12, the end of Week 24, and the end of Week 28.

[21] Color, appearance, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, and occult blood. Microscopic inspection of sediment should only be performed for cause or to investigate an abnormal dipstick finding per the Investigator's discretion. Urinalysis will be performed at Screening, prior to dosing on Day 1, at the end of Week 2, the end of Week 12, the end of Week 24, and the end of Week 28.

[22] Must be repeated at any point throughout the study period if pregnancy is clinically suspected.

[23] Total cholesterol, HDL-C, and triglyceride samples will be collected in the morning following an overnight fast.

[24] Three Screening/Baseline samples will be collected for estradiol and testosterone (total and free). Samples will be collected in the AM on 3 different days; the samples collected on Baseline/Day 1 may comprise one of these; the Screening samples may be collected at any 2 time points during Screening at least 2 days apart. Serum estrone, estradiol, and free and total testosterone will then follow the schedule indicated on Day 1 and Weeks 12, 24, and 28. FSH will only be performed at Screening for female patients only for confirmation of post-menopausal status.

[25] The second Screening hormone testing will consist of estradiol and testosterone (free and total) only. Samples should be drawn in the AM (does not need to be fasting), and may be drawn at the same time the patient returns for the second CBC sample.

[26] Serum osteocalcin-N-mid and CTX will be drawn in the AM each time, approximately between 8-10 AM, and after an overnight fast of 10-12 hours.

[27] Randomization will be performed following PKR genotyping and prior to and as close as feasible to dosing on Day 1.

[28] Study drug administration is not required on W24/D169 for patients not continuing into the Extension Period.

[29] Study drug will be dispensed on a 28-day schedule, or on an alternate schedule (< 28 days) as needed to accommodate patient visit schedule and dose modifications. The amount of study drug dispensed should be sufficient to provide an adequate reserve supply of AG-348 to ensure uninterrupted dosing in the event of an unexpected delay for the next scheduled study visit (7 extra days of dosing supply is recommended during the Core Period).

[30] For the first 10 patients treated, extensive PK/PD sampling will be conducted on Days 1 and 15 (see protocol Appendix 15.1, Table 7 for details), followed by limited PK/PD sampling from Week 3 to Week 24 (see protocol Appendix 15.1, Table 8 for details). Limited PK/PD sampling will be conducted on the remainder of patients treated (see protocol Appendix 15.1, Table 8). See protocol Section 10.6, Section 10.7, and Section 10.9 for details on blood sampling for PK and PD assessments, respectively, and guidelines on sample processing and storage.

[31] [redacted]

[32] Menstruating female patients will record their menstrual cycles (start, stop, characteristics) monthly. Paper-based menstrual cycle diaries will be dispensed at study visits approximately every month. The previous month's diary will be collected at these visits as well.

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

[34] All transfusions must be recorded in the eCRF

[35] Patient must have completed 24 weeks of treatment and tolerated AG-348 (may have had dose modifications). Investigator and Medical Monitor or Responsible Medical Officer must agree with the patient continuing on treatment and patient must sign a separate ICF for the Extension Period.

Table 4-2: Schedule of Assessments: Extension Period

Visit	Month 9	Month 12	Month 15	Month 18	Month 21	Month 24	Month 27	Month 30	Follow-up
Approximate Study Day	**259**	**349**	**439**	**529**	**619**	**709**	**799**	**889**	**919**
Visit Window	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**
Physical Examination/Weight[1]	X	X	X	X	X	X	X	X	X
Performance Status	X	X	X	X	X	X	X	X	X
Vital signs (BP, HR, RR, T)[2]	X	X	X	X	X	X	X	X	X
12-lead ECG[3]		X		X		X		X	X
DXA Scan				X				X	
Laboratory Evaluations[4]									
Hematology (CBC)[5]	X	X	X	X	X	X	X	X	X
Haptoglobin		X		X		X		X	X
EPO levels[6]		X		X		X		X	X
Hepcidin		X		X		X		X	
Serum Chemistry[7]	X	X	X	X	X	X	X	X	X
Iron Panel[8]		X		X		X		X	
Carboxyhemoglobin (COHb)[9]		X		X		X		X	
Coagulation Studies[10]	X	X	X	X	X	X	X	X	X
Urinalysis[11]	X	X	X	X	X	X	X	X	X
Serum or Urine Pregnancy[12]									
Lipids[13]	X	X	X	X	X	X	X	X	X
Hormonal Testing[14]	X	X	X	X	X	X	X	X	X
Serum osteocalcin-N-mid and CTX[15]				X				X	

Visit	Month 9	Month 12	Month 15	Month 18	Month 21	Month 24	Month 27	Month 30	Follow-up
Approximate Study Day	**259**	**349**	**439**	**529**	**619**	**709**	**799**	**889**	**919**
Visit Window	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**	**± 2 W**
Study Drug Administration	X	X	X	X	X	X	X	X	
Dispense Study Drug[16]	X	X	X	X	X	X	X		
PK blood sampling[17]	X	X	X	X	X	X	X	X	
PD Assessments[18] (2,3-DPG/ATP, [redacted])	X	X	X	X	X	X	X	X	
Dispense/Collect Menstrual Cycle Diary[19]	X	X	X	X	X	X	X	X	X
Adverse Events[20]	Continuous								X
Transfusion Record[21]	X	X	X	X	X	X	X	X	X
Concomitant Medications/Procedures	X	X	X	X	X	X	X	X	X

Abbreviations: ATP = adenosine triphosphate; BP = blood pressure; CBC= complete blood count; COHb = carboxyhemoglobin; CTX = C-terminal telopeptide; DPG = diphosphoglycerate; DXA = Dual-energy x-ray absorptiometry; ECG = electrocardiogram; EPO = erythropoietin; HDL-C = high-density lipoprotein-cholesterol; HIV = human immunodeficiency virus; HR = heart rate; PD = pharmacodynamic; PK = pharmacokinetic; PK deficiency = pyruvate kinase deficiency; PKR = pyruvate kinase isoform R; RR = resting rate; W = week.

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

[1] A focused physical examination (including neurological examination; genital and rectal examinations will be performed at the discretion of the Investigator) will be obtained every 3 months; a complete physical examination will be performed at the Month 30 visit. The neurological examination must include an assessment of general orientation and mental status including level of alertness (assess as normal or abnormal and specify any abnormality).

[2] Vital signs will include systolic and diastolic blood pressure, heart rate, respiratory rate, and temperature

[3] 12-lead ECGs are to be conducted after 5 minutes of recumbency.

[4] Laboratory evaluations (hematology, serum chemistry, coagulation studies, and urinalysis) are to be collected in the morning. These should be collected following an overnight fast.

[5] CBC will include HCT, Hb, red blood cell (RBC) count, absolute reticulocyte count, percent reticulocyte count, mean corpuscular volume (MCV), mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count with automated (or manual, as indicated) differential, absolute neutrophil count (ANC), absolute lymphocyte count (ALC), and platelet count. In the event that the designated central laboratory for the study is unable to provide a valid result for any specific component of the defined CBC for a specific patient, the site may be asked

to have the test performed at their local laboratory. The result of the local CBC will be entered into the study database along with the local normal reference range.

[6] Erythropoietin (EPO) levels will be performed prior to dosing at Month 12, Month 18, Month 24, and Month 30.

[7] Alkaline phosphatase, sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide (CO2) or bicarbonate, albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, uric acid, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin and indirect bilirubin.

[8] Iron, total iron-binding capacity (TIBC), transferrin saturation, and ferritin will be performed prior to dosing at Month 12, Month 18, Month 24, and Month 30.

[9] To be collected before the AG-348 morning dose is administered.

[10] Fibrinogen, activated partial thromboplastin time (aPTT), and international normalized ratio (INR) will be performed at each study visit.

[11] Color, appearance, pH, specific gravity, ketones, protein, glucose, bilirubin, nitrite, urobilinogen, and occult blood. Microscopic inspection of sediment should only be performed for cause or to investigate an abnormal dipstick finding per the Investigator's discretion. Urinalysis will be performed prior to dosing at each study visit.

[12] Must be obtained at any point throughout the study period if pregnancy is clinically suspected. Effective contraception must be continued throughout the Extension Period in all patients and any pregnancies must be reported (see protocol Section 11.3).

[13] Total cholesterol, HDL-C, and triglyceride samples will be collected in the morning following an overnight fast.

[14] Serum estrone, estradiol, and free and total testosterone.

[15] Serum osteocalcin-N-mid and CTX will be drawn in the AM each time, approximately between 8-10 AM, and after an overnight fast of 10-12 hours.

[16] Study drug will be dispensed on a 3-month schedule, or on an alternate schedule (< 3 months) as needed to accommodate patient visit schedule and dose modifications. The amount of study drug dispensed should be sufficient to provide an adequate reserve supply of AG-348 to ensure uninterrupted dosing in the event of an unexpected delay for the next scheduled study visit (14 extra days of dosing supply is recommended during the Extension Period).

[17] Predose; PK sampling will only include AG-348 and AGI-8702 concentrations.

[18] Predose.

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

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

[21] All transfusions must be recorded in the eCRF.

Appendix B Preferred Reporting Units

Table 12-1 Clinical Laboratory Preferred Reporting Units

Clinical Laboratory	Standard Unit	Original/Preferred Unit
Haemoglobin	g/L	g/dL
Bilirubin Indirect	umol/L	mg/dL
Bilirubin total	umol/L	mg/dL
Ferritin	pmol/L	ng/mL
Iron	umol/L	ug/dL
Free Testosterone	pmol/L	pg/mL
Total Testosterone	nmol/L	pg/mL
Triglycerides	mmol/L	mg/dL
Cholesterol	mmol/L	mg/dL
HDL-C	mmol/L	mg/dL

Appendix C Imputation Rules for Missing Prior/Concomitant Medication Dates

Imputation rules for missing or partial medication start/stop dates are defined below:

1. Missing or partial medication start date:
    a. If only DAY is missing, use the first day of the month.
    b. If DAY and Month are both missing, use the first day of the year.
    c. If DAY, Month and Year are all missing, use a date before the first dose date.
2. Missing or partial medication stop date:
    a. If only DAY is missing, use the last day of the month.
    b. If DAY and Month are both missing, use the last day of the year.
    c. If DAY, Month and year are all missing, assign 'continuing' status to stop date.

In summary, the prior, concomitant or post categorization of a medication is described below.

Table 12-2 Prior, Concomitant, and Post Categorization of a Medication

	Medication Stop Date		
Medication Start Date	**< First Dose Date of Study Drug**	**≥ First Dose Date and ≤ End Date of Treatment-Emergent Period**	**> End Date of Treatment-Emergent Period**
< First dose date of study drug	P	PC	PCA
≥ First dose date and ≤ End date of Treatment-Emergent period	-	C	CA
> End date of Treatment-Emergent period	-	-	A

A: Post; C: Concomitant; P: Prior

Appendix D Handling of Missing Dates in Adverse Events

For AEs with missing or partial start dates, if there is no clear evidence that the AEs started before the corresponding study period as appropriate, then the AEs will be classified as TEAEs.

As an intermediate step for programming purpose, imputation rules for missing or partially missing AE start/end dates are defined below.

If Year of AE start date is missing:

If year of the AE start date is missing, then compare the full (or partial) AE end date to the first dose date. If the AE end date is before the first dose date then the AE should be considered as a pretreatment AE. Otherwise, the AE will be considered as TEAE.

If Year of AE start date is not missing:

<u>If both day and month of the AE start date are missing:</u>

- If year of the AE start date = the year of the first dose date, impute the missing day and month of the AE start date using the day and month of the first dose date. If this leads to a date after the AE end date, then impute the missing day and month of the AE start date using the day and month of the AE end date. Note:
    - If the AE ended before first dose date, then the AE will be classified as a pre-treatment AE. If the AE ended after the first dose date, then there is not enough evidence to determine whether the AE is a TEAE or not, and to be conservative, the AE will be classified as a TEAE.
- If year of the AE start date ≠ the year of the first dose date, impute the missing day and month of the AE start date using the 1 and Jan.
    - If the year is before the year of the first dose date, then by imputing the missing day and month of the AE start date January 1, TEAE status will be pre-treatment.
    - If the year is after the year of the first dose date, then by imputing the missing day and month of the AE start date January 1, TEAE status will be TEAE if AE start date is on or before the last dose date +28 days; and will be post-treatment AE start date is after the last dose date+28 days.

<u>If only day of the AE start date is missing:</u>

- If the year of the AE start date = the year of the first dose date = the year of the AE end date, and the month of the AE start date = the month of the first dose date = the month of the AE end date, then impute the missing day of AE start date using the smaller non-missing value of (day of the first dose, day of the AE end date).
- If (the year of the AE start date = the year of the first dose date), and (the month of the AE start date = the month of the first dose date), and (the year of the AE start date < the year of the AE end date, or the month of the AE start date < the month of the AE end date), then impute the missing day of AE start date using the day of the first dose.

- If (the year of the AE start date = the year of the first dose date), and (the month of the AE start date = the month of the first dose date), and the AE is ongoing, then impute the missing day of AE start date using the day of the first dose.
- Otherwise, impute the missing day of the AE start date as 1.

Missing or partially missing AE stop date will not be imputed.

Appendix E PKR genotype information and the corresponding mutation description and classification

Due to the evolving understanding on the PKR genotype, the mutations are mostly entered into eCRF as free text. These mutations have been reviewed by the team and the following mapping of each PKR genotype is mapped to the corresponding mutation description and classifications.

Table 1 PKR genotype information and corresponding mutation description and classification

Mutation	Mutation description	Mutation classification
1003 G>A	V335M	Missense
1008 A>AA	insertion (frameshift)	non-missense
1010 G>A	R337Q	Missense
1022 G>C	G341A	Missense
1072 G>A	G358R	Missense
1091 G>A	G364D	Missense
1151 C>T	T384M	Missense
1153 A>T	R385W	Missense
1178 A>G	N393S	Missense
1178 A>G, exon4IVS-IV+10G>T	N393S/splicing	Non-missense
1179 T>A	N393K	Missense
1223 C>T	T408I	Missense
1228 A>G	K410E	Missense
1318 G>T	truncation	Non-missense
1373 G>A	G458D	Missense
142_159del	in-frame deletion	Non-missense
1435 C>T	R479C	Missense
1436 G>A	R479H	Missense
1442 C>T	A481V	Missense
1456 C>T	R486W	Missense
1463 G>A	R488Q	Missense
1483 G>A	A495T	Missense
1484 C>T	A495V	Missense
1487 T>G	V496G	Missense
1493 G>A	R498H	Missense
1528 C>T	truncation (R510stop)	Non-missense
1529 G>A	R510Q	Missense
1574 G>A	truncation	Non-missense
1574 G>GG (1612 G>GG for site [redacted])*	insertion (frameshift)	Non-missense
1594 C>T (1633 CGG>TGG for site [redacted])*	R532W	Missense
284-2A>C	splicing	Non-missense

307delC	in-frame deletion	Non-missense
376-2A>C	splicing	Non-missense
389 C>A	S130Y	Missense
391_393delATC	in-frame deletion	Non-missense
401 T>A	V134D	Missense
494 G>T	G165V	Missense
507+1G>A	splicing	Non-missense
664 G>A	G222R	Missense
695-3C>G	splicing	Non-missense
-70 A>C	splicing	Non-missense
721 G>T (760 GAG>TAG or 760 G>T for site [redacted])*	truncation	Non-missense
721 G>T, 826delG	truncation/frameshift	Non-missense
92 C>T (131 GCT>GTT or 131 C>T for site [redacted])*	A31V	Missense
953_955delAAG	in-frame deletion	Non-missense
exon10del	deletion	Non-missense
exons3-9del	deletion	Non-missense
*Site [redacted] used a different nomenclature to label the mutations (as indicated and reviewed by Translational Science)		

Appendix F MedDRA Terms List for AEs of Endocrinological Interest (Menopausal Symptoms)

Based on AG-348-C-003 List of Terms for analysis of Menopausal Symptoms Version 1.0 (MedDRA Version 20.0) Dated 28Apr2017.

List of PTs for Menopause Sypntom:

HLT	PTs	Code
Menstruation and uterine bleeding NEC	Abnormal withdrawal bleeding	10027335
	Anovulatory cycle	
	Bleeding anovulatory	
	Delayed menarche	
	Dysfunctional uterine bleeding	
	Dysmenorrhoea	
	Menstrual discomfort	
	Menstrual disorder	
	Menstruation irregular	
	Metrorrhagia	
	Premature menarche	
	Premenstrual cramps	
	Premenstrual dysphoric disorder	
	Premenstrual headache	
	Premenstrual pain	
	Premenstrual syndrome	
	Retrograde menstruation	
	Withdrawal bleed	
Menstruation with decreased bleeding	Amenorrhoea	10027341
	Hypomenorrhoea	
	Menstruation delayed	
	Oligomenorrhoea	
	Pituitary amenorrhoea	
Menstruation with increased bleeding	Menometrorrhagia	10027342
	Menorrhagia	
	Polymenorrhagia	
	Polymenorrhoea	
Emotional and mood disturbances NEC	Mood swings	10027951
Other PTs	Hot flush	10060800

HLTs are too broad	Menopausal symptoms	10027304
	Premature menopause	10036601
	Acne	10000496
	Flushing	10016825
	Erectile dysfunction	10061461
	Arthralgia	10003239
	Arthritis	10003246
	Alopecia	10001760
	Vulvovaginal dryness	10047791
	Vulvovaginal discomfort	10047786
	Dysphoria	10013954
	Night sweats	10013954
	Mood altered	10027940
	Premenstrual dysphoric disorder	10051537
	Premenstrual syndrome	10036618

APPROVAL SIGNATURE PAGE

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

Protocol Number: **AG348-C-003**

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