CLINICAL STUDY PROTOCOL AG348-C-006

A PHASE 3, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY TO EVALUATE THE EFFICACY AND SAFETY OF AG-348 IN NOT REGULARLY TRANSFUSED ADULT SUBJECTS WITH PYRUVATE KINASE DEFICIENCY

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This study will be conducted according to the protocol and in compliance with Good Clinical Practices (GCP) as described in the International Council for Harmonisation (ICH) of Technical Requirements for Pharmaceuticals for Human Use guidelines, the ethical principles stated in the Declaration of Helsinki, and other applicable regulatory requirements.

PROTOCOL APPROVAL SIGNATURE PAGE
SPONSOR: AGIOS PHARMACEUTICALS, INC.

I hereby approve this clinical study protocol on behalf of Agios Pharmaceuticals, Inc. (Agios/the Sponsor) and attest that it complies with all applicable regulations and guidelines. In addition, I agree that the Investigators participating in this study will be informed of all relevant information that becomes available during the conduct of the study.

Approved by:

DocuSigned by:
[redacted]
Signer Name: [redacted]
Signing Reason: I approve this document
Signing Time: 14-Aug-2019 | 5:08 PM EDT
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14-Aug-2019 | 5:08 PM EDT

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

Date (DD MMM YYYY)

INVESTIGATOR'S AGREEMENT

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

This study will not commence without the prior written approval of a properly constituted Institutional Review Board (IRB)/Independent Ethics Committee (IEC). No changes will be made to the study protocol without the prior written approval of Agios and the IRB/IEC, 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)

Investigational site or name of institution and location (printed)

2. SYNOPSIS

Name of Sponsor/Company:
Agios Pharmaceuticals, Inc.

Name of Investigational Product:
AG-348

Title of Study:
A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of AG-348 in Not Regularly Transfused Adult Subjects With Pyruvate Kinase Deficiency

Study Center(s):
This is a multicenter study that will be conducted in multiple countries.

Phase of Development:
3

Objectives:

Primary:

- To evaluate the efficacy of treatment with AG-348 compared with placebo in increasing hemoglobin (Hb) concentrations

***Secondary*:**

- To evaluate the safety of AG-348
- To determine the effect of the study treatment regimens on markers of hemolysis, hematopoietic activity, and other indicators of clinical activity
- To determine the effect of the study treatment regimens on health-related quality of life (HRQoL), as determined using patient-reported outcomes (PROs)
- To evaluate the pharmacokinetics of AG-348 after oral administration
- To evaluate the relationship between AG-348 pharmacokinetics and safety parameters

***Exploratory*:**

- To evaluate the relationship of AG-348 pharmacokinetics to indicators of clinical activity
- To evaluate the pharmacodynamic markers of pyruvate kinase deficiency (PK deficiency) and how they are affected by study treatment
- To determine the effect of the study treatment regimens on:
  - Number of transfusion events and number of red blood cell (RBC) units transfused
  - Markers of iron metabolism and indicators of iron overload

Methodology:

Overview:

This is a Phase 3, randomized, multicenter, double-blind, placebo-controlled study consisting of a Dose Optimization Period (Part 1) followed by a Fixed Dose Period (Part 2) (see study schema below).

After a Screening Period, eligible subjects will be randomized 1:1 to receive either AG-348 or matching placebo. The term "study treatment" is used throughout the protocol to define both the active (AG-348) drug and placebo. Randomization will be stratified by the average of screening Hb concentrations (<8.5 vs ≥8.5 g/dL [5.28 mmol/L]) and the *PKLR* gene mutation category (missense/missense vs missense/non-missense). In rare instances in which *PKLR* gene mutation

category cannot be made definitively (eg, if a subject harbors 3 mutant *PKLR* alleles), the subject will be assigned to the missense/non-missense category.

Following randomization to either AG-348 or matching placebo, subjects will enter the Part 1, a 12-week period starting on Day 1 of the study. The goal of the Dose Optimization Period is to maximize a subject's increase in Hb while maintaining an acceptable safety profile. All subjects will receive an initial dose of 5 mg twice daily (BID) of study treatment with 2 potential sequential steps for dose level increase (ie, from 5 to 20 mg BID and from 20 to 50 mg BID; no increases beyond 50 mg BID will be allowed). The first dose of study treatment on Day 1 should be taken at the study site following all Day 1 assessments (with the exception of electronic diary [eDiary] assessments).

Subjects will be assessed for safety and efficacy (as defined by Hb increase) every 4 weeks during Part 1, to determine if their dose should be increased, maintained at the current level, or decreased. At the Week 4 and Week 8 Visits, study treatment dose should be increased to the next dose level if the subject has met both of the following criteria:

- The subject is tolerating the study treatment, **and**
- The subject's Hb concentration on the day of the visit based on local laboratory results is lower than 2.5 g/dL (1.55 mmol/L) below the upper limit of normal (ULN), as applies to men and women.

Dose re-escalation or re-introduction should be avoided after the Week 8 Visit, but may be permitted, after discussion with the Independent Medical Monitor, or designee.

At the Week 12 Visit, if the subject has tolerated the study treatment, the subject will remain at his/her current dose level. If the Investigator deems it necessary to reduce the study treatment for safety reasons, the subject's dose may be reduced to 1 of the 2 available lower dose levels (ie, 5 mg BID, 20 mg BID). If the subject is already receiving 5 mg BID and/or cannot tolerate BID dosing, another regimen may be allowed after discussion with, and approval by, the Independent Medical Monitor, or designee.

If questions arise about whether the dose level of a given subject should be increased, maintained, or decreased, the Independent Medical Monitor, or designee, should be contacted.

At any time during the study, the Investigator can discontinue, interrupt, or reduce the subject's dose of study treatment for reasons related to safety.

Following Part 1, each subject will remain on his/her individually optimized dose and enter the 12-week Fixed Dose Period (Part 2). For the purposes of dosing during the Fixed Dose Period, the dose the subject is being administered at the Week 12 Visit will be considered the subject's optimized dose and will be the dose the subject receives during Part 2.

All subjects who remain on study during Part 2 through the Week 24 Visit may be eligible for an open-label extension study, in which all subjects will receive AG-348.

All subjects who discontinue or interrupt study treatment during the study should undergo the recommended dose taper, unless an emergency situation justifies discontinuing or interrupting the study treatment abruptly. Whether or not the recommended dose taper is performed, subjects who discontinue or interrupt study treatment should be monitored as clinically indicated for signs of withdrawal hemolysis and worsening of anemia.

Visit Schedule and Analysis Time Points:

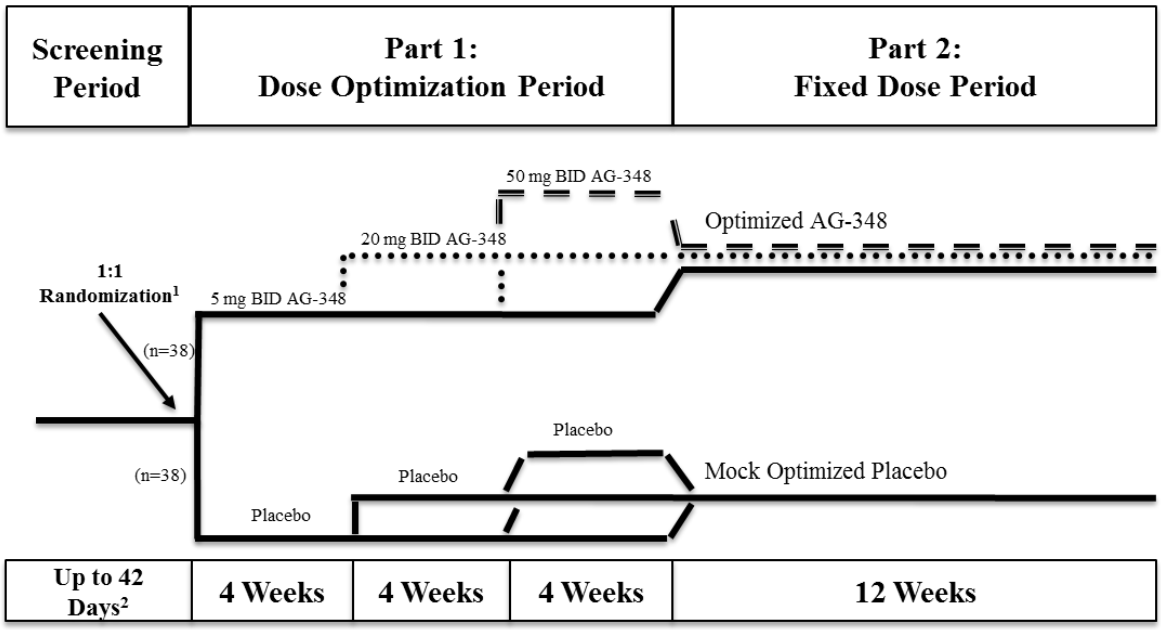
A study schema is presented in the figure below.

The Screening Period will last up to 42 days (a subject's Screening Period duration may be extended beyond 42 days upon the Medical Monitor's, or designee's, approval). During Part 1, all subjects will attend visits on Day 1, Week 2, Week 4, Week 6, Week 8, Week 10, and Week 12. During Part 2, all subjects will attend visits every 4 weeks through Week 24 (ie, Week 16, Week 20, and Week 24).

Subjects who discontinue study prior to the Week 24 Visit should attend the End of Study Visit, but do not need to complete the Follow-Up Visit. Subjects who continue the study through the Week 24 Visit on study treatment but who do not continue on into an extension study, will attend the Follow-up Visit. Subjects who continue on into an extension study at the Week 24 Visit will not be required to attend the Follow-up Visit.

All adverse events (AEs) will be monitored until resolution of the AE to baseline, the AE is considered stable within the context of the trial, the subject is lost to follow-up, or until 28 days after the last dose of study treatment unless the subject is enrolled in an extension study, in which case ongoing AEs will be reported in the extension study database following consenting of the subject. All serious adverse events (SAEs) will be followed until final outcome of the SAE is known, the subject is lost to follow-up, or the subject enrolls in an extension study, in which case the SAE follow-up information will be reported in the extension study database.

Study Schema



Abbreviations: BID = twice daily.
[1]Stratified by average of screening Hb concentrations (Hb <8.5 g/dL vs Hb ≥8.5 g/dL [5.28 mmol/L]) and the *PKLR* gene mutation category (missense/missense vs missense/non-missense)
[2] A subject's Screening Period duration may be extended beyond 42 days upon the Medical Monitor's, or designee's, approval.

Number of Subjects (Planned):

Approximately 76

Diagnosis and Main Criteria for Inclusion:

Inclusion Criteria:

For enrollment into this study, subjects must meet all of the following criteria during the Screening Period:

1. Have provided signed written informed consent prior to performing any study procedure, including screening procedures.
2. Be aged 18 years or older.

3. Have documented clinical laboratory confirmation of PK deficiency, defined as documented presence of at least 2 mutant alleles in the *PKLR* gene, of which at least 1 is a missense mutation, as determined per the genotyping performed by the central genotyping laboratory.
4. Have an Hb concentration less than or equal to 10.0 g/dL (6.21 mmol/L) regardless of gender (average of at least 2 Hb measurements [separated by a minimum of 7 days] during the Screening Period).
5. Be considered not regularly transfused, defined as having had no more than 4 transfusion episodes in the 12-month period up to the first day of study treatment **and** no transfusions in the 3 months prior to the first day of study treatment.
6. Have received at least 0.8 mg oral folic acid daily for at least 21 days prior to the first dose of study treatment, to be continued daily during study participation.
7. Have adequate organ function, as defined by:
    a. Serum aspartate aminotransferase (AST) $\leq 2.5 \times$ ULN (unless the increased AST is assessed by the Investigator as due to hemolysis and/or hepatic iron deposition) and alanine aminotransferase (ALT) $\leq 2.5 \times$ ULN (unless the increased ALT is assessed by the Investigator as due to hepatic iron deposition).
    b. Normal or elevated levels of serum bilirubin. In subjects with serum bilirubin >ULN, the elevation must not be associated with choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease. Elevated bilirubin attributed to hemolysis with or without Gilbert's syndrome is not exclusionary.
    c. Estimated glomerular filtration rate (GFR) ≥ 60 mL/min/1.73 m^2, measured GFR ≥ 60 mL/min, or calculated creatinine clearance (CrCL; Cockcroft-Gault) ≥ 60 mL/min.
    d. Absolute neutrophil count $\geq 1.0 \times 10^9$/L (based on an average of at least 2 measurements [separated by a minimum of 7 days] during the Screening Period).
    e. Platelet count $\geq 100 \times 10^9$/L in the absence of a spleen, or platelet count $\geq 50 \times 10^9$/L in the presence of a spleen and in the absence of any other cause of thrombocytopenia (based on an average of at least 2 measurements [separated by a minimum of 7 days] during the Screening Period).
    f. Activated partial thromboplastin time and international normalized ratio $\leq 1.25 \times$ ULN, unless the subject is receiving therapeutic anticoagulants.
8. For women of reproductive potential, have a negative serum pregnancy test during the Screening Period. Women of reproductive potential are defined as sexually mature women who have not undergone a hysterectomy, bilateral oophorectomy, or tubal occlusion; or who have not been naturally postmenopausal (ie, who have not menstruated at all for at least the preceding 12 months prior to signing informed consent and have an elevated follicle-stimulating hormone level indicative of menopause during the Screening Period).
9. For women of reproductive potential as well as men with partners who are women of reproductive potential, be abstinent as part of their usual lifestyle, or agree to use 2 forms of contraception, 1 of which must be considered highly effective, from the time of giving informed consent, during the study, and for 28 days following the last dose of study treatment for women and 90 days following the last dose of study treatment for men. A highly effective form of contraception is defined as combined (estrogen and progestin containing) hormonal contraceptives (oral, intravaginal, or transdermal) known to be associated with inhibition of ovulation; progestin-only hormonal contraceptives (oral, injectable, or implantable) known to be associated with inhibition of ovulation; intrauterine device; intrauterine hormone releasing system; bilateral tube occlusion; or vasectomized partner. The second form of contraception can include an acceptable barrier method, which includes male or female condoms with or without spermicide, and cervical cap, diaphragm, or sponge with spermicide. Women of

reproductive potential using hormonal contraception as a highly effective form of contraception must also utilize an acceptable barrier method while enrolled in the study and for at least 28 days after their last dose of study treatment.

10. Be willing to comply with all study procedures for the duration of the study.

Exclusion Criteria:

Subjects who meet any of the following criteria during screening will not be enrolled in the study:

1. Are homozygous for the R479H mutation or have 2 non-missense mutations, without the presence of another missense mutation, in the *PKLR* gene as determined per the genotyping performed by the central genotyping laboratory.
2. Have a significant medical condition that confers an unacceptable risk to participating in the study, and/or that could confound the interpretation of the study data. Such significant medical conditions include, but are not limited to the following:
   a. Poorly controlled hypertension (defined as systolic blood pressure [BP] >150 mmHg or diastolic BP >90 mmHg) refractory to medical management.
   b. History of recent (within 6 months prior to signing informed consent) congestive heart failure; myocardial infarction or unstable angina pectoris; hemorrhagic, embolic, or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.
   c. Cardiac dysrhythmias judged as clinically significant by the Investigator.
   d. Heart-rate corrected QT interval-Fridericia's method (QTcF) >450 msec (average of triplicate electrocardiograms [ECGs]) with the exception of subjects with right or left bundle branch block.
   e. Clinically symptomatic cholelithiasis or cholecystitis. Prior cholecystectomy is not exclusionary. Subjects with symptomatic cholelithiasis or cholecystitis may be rescreened once the disorder has been treated and clinical symptoms have resolved.
   f. History of drug-induced cholestatic hepatitis.
   g. Iron overload sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac (eg, clinically significant impaired left ventricular ejection fraction), hepatic (eg, fibrosis, cirrhosis), or pancreatic (eg, diabetes) dysfunction.
   h. Have a diagnosis of any other congenital or acquired blood disorder, or any other hemolytic process, except mild allo-immunization as a consequence of transfusion therapy. Genetic findings that in isolation are predicted to be insufficient to explain the observed clinical phenotype may be allowed (eg, heterozygous status for certain recessive red blood cell disorders).
   i. Positive test for hepatitis B surface antigen or hepatitis C virus (HCV) antibody (Ab) with signs of active hepatitis B or C virus infection. If the subject is positive for HCVAb, a reverse transcriptase-polymerase chain reaction test will be conducted. Subjects with hepatitis C may be rescreened after receiving appropriate hepatitis C treatment.
   j. Positive test for human immunodeficiency virus (HIV)-1 or -2 Ab.
   k. Active infection requiring the use of parenteral antimicrobial agents or Grade ≥3 in severity (per National Cancer Institute Common Terminology Criteria for Adverse Events) within 2 months prior to the first dose of study treatment.
   l. Diabetes mellitus judged to be under poor control by the Investigator or requiring >3 antidiabetic agents, including insulin (all insulins are considered 1 agent); use of insulin per se is not exclusionary.
   m. History of any primary malignancy, with the exception of: curatively treated nonmelanomatous skin cancer; curatively treated cervical or breast carcinoma in situ; or

other primary tumor treated with curative intent, no known active disease present, and no treatment administered during the last 3 years.

n. Unstable extramedullary hematopoiesis that could pose a risk of imminent neurologic compromise.

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

3. Have a splenectomy scheduled during the study treatment period or have undergone splenectomy within 12 months prior to signing informed consent.
4. Are currently enrolled in another therapeutic clinical trial involving ongoing therapy with any investigational or marketed product or placebo. Prior and subsequent participation in the PK Deficiency Natural History Study (NHS) (NCT02053480) or PK Deficiency Registry is permitted, however, concurrent participation is not. Therefore, subjects enrolling in this current study will be expected to temporarily suspend participation in the NHS or Registry.
5. Have exposure to any investigational drug, device, or procedure within 3 months prior to the first dose of study treatment.
6. Have had any prior treatment with a pyruvate kinase activator.
7. Have a prior bone marrow or stem cell transplant.
8. Are currently pregnant or breastfeeding.
9. Have a history of major surgery within 6 months of signing informed consent. Note that procedures such as laparoscopic gallbladder surgery are not considered major in this context.
10. Are currently receiving medications that are strong inhibitors of cytochrome P450 (CYP)3A4, strong inducers of CYP3A4, strong inhibitors of P-glycoprotein (P-gp), or digoxin (a P-gp sensitive substrate medication) that have not been stopped for a duration of at least 5 days or a timeframe equivalent to 5 half-lives (whichever is longer) prior to the first dose of study treatment.
11. Are currently receiving hematopoietic stimulating agents (eg, erythropoietins, granulocyte colony stimulating factors, thrombopoietins) that have not been stopped for a duration of at least 28 days prior to the first dose of study treatment.
12. Have a history of allergy to sulfonamides if characterized by acute hemolytic anemia, drug-induced liver injury, anaphylaxis, rash of erythema multiforme type or Stevens-Johnson syndrome, cholestatic hepatitis, or other serious clinical manifestations.
13. Have a history of allergy to AG-348 or its excipients (microcrystalline cellulose, croscarmellose sodium, sodium stearyl fumarate, and mannitol).
14. Are currently receiving anabolic steroids, including testosterone preparations, within 28 days prior to the first dose of study treatment.

Investigational Product, Dosage, and Mode of Administration:

AG-348 will be administered orally BID as tablets of different sizes for the 5, 20, and 50 mg dose levels. Subjects will be receiving 1 of 3 potential doses, each of which is supplied as a different sized tablet. Thus, to maintain blinding, each dose of study treatment will be supplied as 3 different sized tablets: 1 tablet will be the active drug and the other 2 tablets will be placebo for subjects who are randomized to active, and all 3 tablets will be placebo for subjects who are randomized to placebo.

Doses of AG-348 may be taken with or without food.

Subjects should be advised not to discontinue or interrupt dosing without first speaking with the treating Investigator except in case of medical emergency; abrupt discontinuation or interruption of AG-348 may result in withdrawal hemolysis. If a subject needs to discontinue or interrupt study treatment at any time during the study, guidance is provided.

Reference Therapy, Dosage, and Mode of Administration:

Placebo will be administered as 3 visually matched tablets of different sizes, and dosed following the same guidelines and procedures as active treatment.

Duration of Study Treatment (ie, AG-348/Matching Placebo):

The duration of study treatment will be 24 weeks (not including the recommended dose taper).

End of Study:

End of Study is defined as the time at which all subjects have completed the study or are lost to follow-up.

Study Endpoints:

Primary Endpoint:

The primary endpoint is the hemoglobin response (HR), defined as a ≥1.5 g/dL (0.93 mmol/L) increase in Hb concentration from baseline that is sustained at 2 or more scheduled assessments at Weeks 16, 20, and 24 during the Fixed Dose Period. The individual subject's baseline Hb concentration is defined as the average of all available Hb concentrations collected for that subject during the Screening Period up to the first dose of study treatment.

Key Secondary Endpoint:

- Average change from baseline in Hb concentration at Weeks 16, 20, and 24

Other Secondary Endpoints:

- Maximal Hb concentration increase from baseline
- Time to first achieve an increase in Hb concentration of 1.5 g/dL (0.93 mmol/L) or more from baseline
- Average change from baseline at Weeks 16, 20, and 24 in markers of hemolysis: bilirubin, lactate dehydrogenase, and haptoglobin levels
- Average change from baseline at Weeks 16, 20, and 24 in markers of hematopoietic activity: reticulocyte percentages
- Change from baseline in HRQoL PRO scores: Pyruvate Kinase Deficiency Diary and Pyruvate Kinase Deficiency Impact Assessment
- Safety endpoints, including: the type, incidence, severity, and relationship to study treatment of AEs and serious adverse events (SAEs); number of discontinuations due to AEs; results of clinical laboratory tests over time (eg, serum chemistry, liver function test, hematology, lipids, sex steroids, urinalysis, coagulation); physical examination findings; dual-energy x-ray absorption (DXA) scans, vital signs; 12-lead ECG data

- Pharmacokinetic endpoints, including plasma concentrations over time and pharmacokinetic parameters of AG-348 (eg, area under the concentration × time curve [AUC], maximum [peak] concentration [C_{max}], others as applicable)
- Exposure-response relationship between safety parameters and AG-348 concentration and relevant AG-348 pharmacokinetic parameters

Exploratory Endpoints:

- Exposure-response (or pharmacokinetic-pharmacodynamic) relationship between relevant pharmacokinetic parameters and endpoints that are indicators of clinical activity
  - Change from baseline in additional markers of hematopoietic activity
  - Change from baseline in markers of iron metabolism and indicators of iron overload
  - Change from baseline in the red blood cell-specific form of pyruvate kinase (PKR) protein level
  - Relationship between baseline PKR protein level and Hb response status
  - Change from baseline in HRQoL PRO scores: European quality of life five-dimensional descriptive system (EQ-5D-5L)
  - Change from baseline in PKR flux assay results
  - Proportion of subjects requiring transfusions and the total number of RBC units transfused

Randomization, Blinding, Handling of Restricted Data, and Unblinding:

Randomization: Eligible subjects will be randomized 1:1 to receive either AG-348 or matching placebo. The randomization will be stratified by the average of screening Hb concentrations (<8.5 vs ≥8.5 g/dL [5.28 mmol/L]) and the *PKLR* gene mutation category (missense/missense vs missense/non-missense). The randomization assignment will be double-blinded to minimize any potential assessment bias. In rare instances where *PKLR* gene mutation category cannot be made definitively (eg, if a subject harbors 3 mutant *PKLR* alleles), the subject will be assigned to the missense/non-missense category.

Blinding: The subject, Investigators, and site personnel will be blinded to the subject's study treatment allocation until database lock, if that subject is not entering an extension study. If the subject expresses his/her intention to enter an extension study, the subject, Investigators, and site personnel will be unblinded to the subject's study treatment allocation, but only after the subject completes the Week 24 assessments. The Sponsor study team will be blinded to study treatment allocation until the database has been locked.

Handling of Restricted Data: The following data will be considered restricted data: RBC parameters, hemolysis parameters, and hormone data. The Investigators will have access to these restricted data, with the exception of the hormone data, for their own subjects. Subjects should not have access to their own restricted data, to reduce the potential for bias in the PRO measurements. The Sponsor study team will not have access to the restricted data. The Independent Medical Monitor, or designee, will have access to the restricted data to be able to provide guidance to the Investigators and perform periodic review of these data.

Unblinding: In the event of a medical emergency or pregnancy in a female subject, or in the female sexual partner of a male subject, in which knowledge of the treatment allocation is critical to the subject's management, the blind for that subject may be broken by the Investigator. Prior to unblinding, Investigators are encouraged to discuss a plan to break the blinding code with the Independent Medical Monitor, or designee.

Statistical Methods:

Analysis Sets:

- Full Analysis Set (FAS): All subjects who are randomized. Subjects will be analyzed according to the treatment to which they were randomized, regardless of any errors in dosing. The FAS will be the primary analysis set for the efficacy endpoints.
- Per Protocol Set (PPS): All subjects who are randomized and dosed and have Hb assessments at Weeks 16, 20, and 24 during the Fixed Dose Period. Subjects will be analyzed according to the treatment to which they were randomized, regardless of any errors in dosing. The PPS will be used for a sensitivity analysis for the primary endpoint and the key secondary endpoint.
- Safety Analysis Set (SAS): All subjects who receive at least 1 dose of study treatment. Subjects will be analyzed according to the actual treatment they received (eg, if a subject received any active treatment, the subject will be grouped to the active arm). The SAS will be the primary analysis set for safety analyses.

Statistical Methods:

The study data will be analyzed and reported when all subjects have completed their Week 24 Visit or Follow-up Visit (if applicable) or have discontinued the study. The statistical analysis details will be provided in the Statistical Analysis Plan (SAP), which will be finalized before the database lock and study unblinding.

Study data will be summarized for disposition, demographics, and baseline characteristics (including genotype) based on the FAS.

In the primary efficacy analysis, subjects' HR status will be analyzed using a logistic regression model. The model will include the HR response (Yes vs No) as the dependent variable and treatment as the independent variable, adjusting for stratification factors, including the average of screening Hb concentrations (<8.5 vs ≥8.5 g/dL) and the *PKLR* gene mutation category (missense/missense vs missense/non-missense). The primary analysis will be based on the FAS. In the primary analysis, subjects who discontinue the study before having at least 2 Hb concentration assessments in the Fixed Dose Period will be considered as not achieving an HR. The primary result obtained from the logistic model will be the estimated odds ratio between the active arm vs the placebo arm, along with the 95% confidence interval, and the 2-sided *P* value. If the 2-sided *P* value is less than the required alpha (0.05), then the null hypothesis will be rejected and statistical significance will be claimed for the active arm (ie, AG-348). When the logistic model fails to converge or its maximum likelihood estimate does not exist due to quasi-complete separation, the non-parametric method (ie, Cochran-Mantel-Haenszel test [CMH]) will be used. A sensitivity analysis based on the PPS will be conducted to evaluate the impact of missing assessments due to early study discontinuation. Additional sensitivity and supportive analyses will be specified in the SAP.

The average change from baseline in Hb concentrations at Weeks 16, 20, and 24 will be analyzed and compared between the active arm and the placebo arm by the linear Mixed-Effect Model Repeat Measurement (MMRM) method. The model will include the change in Hb at each visit as the dependent variable; treatment, visit, and treatment-by-visit interaction as fixed effects; and subject as a random effect with adjustment for stratification factors, including the average of screening Hb concentrations (<8.5 vs ≥8.5 g/dL) and the *PKLR* gene mutation category (missense/missense vs missense/non-missense). Based on the least square estimate (LS Means), the average change from baseline of Hb concentrations at Weeks 16, 20, and 24 will be compared between the active arm and the placebo arm. In addition the Hb concentrations and their change from baseline at each visit over time will also be summarized by visit.

Safety will be summarized using descriptive statistics. Summaries will be produced for all treatment-emergent adverse events (TEAEs), related TEAEs (those considered by the Investigator as related to study treatment) TEAEs Grade ≥3 in severity, TEAEs leading to treatment

discontinuation/interruption, SAEs, adverse events of special interest (AESIs), and death. In addition, listings containing individual subject data for all TEAEs leading to treatment discontinuation, TEAEs leading to treatment interruption, serious AEs, AESIs, and death will be provided separately.

For clinical laboratory values, vital signs, and ECG assessments, both actual values and changes from baseline will be summarized by visit using summary statistics. The number and percentage of subjects with transaminase increases of >2.5 × baseline or increases to Grade ≥2, will be summarized.

Interim Analysis and Data Monitoring Committees:

No interim analysis is planned in this study. An Independent Data Monitoring Committee will be established to review study data periodically and provide safety oversight for subjects in the study.

Sample Size:

Assuming a response rate of 35% in the active arm and 5% in the placebo arm, 76 subjects (38 per arm) are needed to have 90% power to detect a treatment effect in HR rate based on a 2-sided Fisher's Exact test at the 0.05 significance level.

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4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
Ab	Antibody
ADP	Adenosine diphosphate
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
AUC	Area under the plasma concentration × time curve
AUC_{0-12}	Area under the plasma concentration × time curve from 0 to 12 hours
AUC_{0-last}	Area under the plasma concentration × time curve from time 0 to the time of the last measurable concentration
BID	Twice daily
BP	Blood pressure
CGIC	Clinician Global Impression of Change
CI	Confidence interval
C_{max}	Maximum (peak) concentration
CrCL	Creatinine clearance
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
CYP	cytochrome P450 enzymes
2,3-DPG	2,3-Diphosphoglycerate
DXA	Dual-energy X-ray absorptiometry
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
eDiary	Electronic diary
EPO	Erythropoietin
EQ-5D-5L	European quality of life five-dimensional descriptive system
FACT-An	Functional Assessment of Cancer Therapy Anemia
FAS	Full Analysis Set
FSH	Follicle-stimulating hormone

Abbreviation	Definition
GCP(s)	Good Clinical Practice(s)
GFR	Glomerular filtration rate
Hb	Hemoglobin
HBsAg	Hepatitis B surface antigen
hCG	Human chorionic gonadotropin
HCT	Hematocrit
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HR	Hemoglobin response
HRQoL	Health-related quality of life
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Council for Harmonisation
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IMP	Investigational medicinal product
INR	International normalized ratio
IRB	Institutional Review Board
IXRS	Interactive response system
LDH	Lactate dehydrogenase
LFT	Liver function test
LIC	Liver iron concentration
LS Means	Least square estimate
MAD	Multiple ascending dose
MCV	Mean corpuscular volume
MMRM	Mixed-Effect Model Repeat Measurement
MRI	Magnetic resonance imaging
NCI	National Cancer Institute
NHS	Natural History Study
NTBI	Non-transferrin bound iron
PD	Pharmacodynamic(s)
PE	Physical examination
PEP	Phosphoenolpyruvate

Abbreviation	Definition
PGIC	Patient Global Impression of Change
PGIS	Patient Global Impression of Severity
P-gp	P-glycoprotein
PK deficient/cy	Pyruvate kinase deficient/cy
PKDD	Pyruvate Kinase Deficiency Diary
PKDIA	Pyruvate Kinase Deficiency Impact Assessment
PKL	Liver-specific form of pyruvate kinase
PKM	Pyruvate kinase muscle isozyme
PKR	Red blood cell-specific form of pyruvate kinase
PPS	Per Protocol Set
PRO	Patient-reported outcome
QD	Once daily
QOD	Every other day
QoL	Quality of Life
QTcB	Heart rate-corrected QT interval by Bazett's method
QTcF	Heart rate-corrected QT interval by Fridericia's method
RBC	Red blood cell
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Safety Analysis Set
SD	Standard deviation
SF-12v2	12-item Short Form Health Survey, Version 2
Study treatment	AG-348 or matching placebo
TEAE	Treatment-emergent adverse event
TIBC	Total iron-binding capacity
T_{max}	Time to maximum (peak) concentration
ULN	Upper limit of normal
WT	Wild-type

5. INTRODUCTION

5.1. Pyruvate Kinase Deficiency

Pyruvate kinase deficiency (PK deficiency) is a glycolytic enzymopathy that results in life-long, nonspherocytic hemolytic anemia. It is an autosomal recessive disease with a variable clinical presentation, ranging from mild to life-threatening, which can be associated with severe, debilitating comorbidities.

5.1.1. Epidemiology and Prevalence

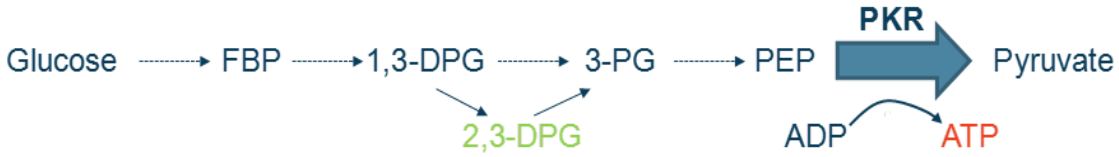
Epidemiological data for PK deficiency are scarce; however, the current estimated diagnosed prevalence of patients with PK deficiency in the United States (US) and European Union 5 (EU5) (ie, France, Germany, Italy, Spain, the United Kingdom) is approximately 2,400 cases (Carey et al, 2000; de Medicis et al, 1992). As with many rare genetic diseases, true prevalence of PK deficiency is not well understood (Beutler and Gelbart, 2000). Most recent estimates have been cited at approximately 1:20,000-1:485,000 (Beutler and Gelbart, 2000; Carey et al, 2000; Hirono et al, 2014; Zanella et al, 2007).

5.1.2. Biochemistry and Genetics

In normal cells, pyruvate kinase enzymatically catalyzes the metabolic conversion of phosphoenolpyruvate (PEP) and adenosine diphosphate (ADP) into pyruvate and adenosine triphosphate (ATP) as the final step in glycolysis. It is believed that PK deficiency leads to insufficient ATP production, resulting in red blood cell (RBC) hemolysis due to an impaired ability to maintain cellular membrane homeostasis (van Wijk and van Solinge, 2005). Indeed, PK deficiency has been reported to be associated with reduced RBC survival, as well as with impaired RBC maturation (Aizawa et al, 2003).

Pyruvate kinase deficiency is the second most common of the glycolytic enzymopathies after glucose-6-phosphate dehydrogenase deficiency. Red blood cells from patients with PK deficiency are characterized by changes in metabolism associated with defective glycolysis, including a deficiency in ATP levels. Levels of 2,3-diphosphoglycerate (2,3-DPG), PEP, and other glycolytic intermediates upstream of the reaction catalyzed by the RBC-specific form of pyruvate kinase (PKR) have been reported to be elevated in patients with PK deficiency, reflecting the inhibition of glycolysis at the PKR step (Oski and Bowman, 1969) (see Figure 1). Red blood cells from patients with PK deficiency show less efficient utilization of glucose than the RBCs of normal healthy individuals (Tanaka et al, 1962).

Figure 1: Glycolysis in Red Blood Cells of Patients With Pyruvate Kinase Deficiency



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-biphosphate; PEP = phosphoenolpyruvate; PKR = red blood cell-specific form of pyruvate kinase.
Note: Not all steps in glycolysis are shown.

Pyruvate kinase deficiency is an autosomal recessive disease (patients must have 2 mutated alleles, usually as compound heterozygotes), and most patients with PK deficiency present with unique combinations of poorly characterized or private mutations. In addition to genetic heterogeneity, PK deficiency exhibits considerable phenotypic variability. For example, the US Pennsylvanian Amish community represents a subgroup of PK deficient patients with a relatively homogeneous genetic background (eg, homozygous R479H mutation, restricted marriage pool) and uniform lifestyle. Yet, despite these similarities, there is still considerable phenotypic variation within this community in terms of disease severity (Grace et al, 2015).

A non-drug study protocol to obtain critical information regarding the natural history of PK deficiency and the range and incidence of related symptoms, treatments, and complications—known as the PK Deficiency Natural History Study (NHS) (Boston Children's Hospital and Agios, 2017) (NCT02053480)—has been developed by Boston Children's Hospital (Boston, Massachusetts, US) and is funded and supported by Agios. This multicenter, global NHS is designed as a longitudinal cohort study with retrospective, baseline, and annual collection of data over a 2-year period. The study has thus far identified 123 mutations in 255 subjects (Bianchi et al, 2017). Fifty mutations, or approximately 40% of the identified mutations, had not been previously described and were only identified as a consequence of the systematic genotyping effort for participants in this NHS. This highlights the evolving understanding of the genetic basis of PK deficiency.

An attempt has been made to impose a system of classification onto this large collection of potential genotypes by dividing the genotypes into 2 classifications and 3 groups (Bianchi et al, 2015). This classification showed that 79 of the 123 mutations (64.2%) were missense mutations, which are single nucleotide changes that result in amino acid substitutions in the PKR enzyme. The effects of these missense mutations can include a loss of catalytic efficiency and/or a loss of protein stability of the enzyme. Non-missense mutations include those that cause premature truncations of the enzyme, deletions or frameshifts, or mutations that affect splicing of the enzyme. Many of these non-missense mutations are predicted to be null alleles of PKR, resulting in a lack of functional protein expression (Bianchi et al, 2017).

5.1.3. Clinical Characteristics

The natural history of untreated PK deficiency is characterized by life-long hemolytic anemia and subsequent associated comorbidities, which can include a need for transfusions, susceptibility to infections after splenectomy, worsening anemia during pregnancy, and symptoms associated with chronic hemolytic anemia (Rider et al, 2011). Some patients with PK deficiency may present with severe hemolytic anemia in early infancy that requires immediate

care. Unconjugated bilirubin is also often chronically elevated in patients with PK deficiency; thus, pigmented gallstones are common in both children and adults with the disease. Additionally, iron overload is progressive and can ultimately lead to life-threatening symptoms.

There are no generally agreed-upon definitions of "mild", "moderate", and "severe" disease, because multiple factors – such as the degree of anemia, the level of bilirubin and severity of jaundice, and complications (including iron overload, transfusion need, and subjective feelings of fatigue and low energy level) – must be taken into account before an evaluation can be made.

There are no guidelines for transfusion management in patients with PK deficiency. Most adults with the disease have been splenectomized and require only sporadic or ad hoc transfusions, which are usually administered in the context of an acute hemolytic episode triggered by infection, trauma, or stress (Grace et al, 2018; Grace et al, 2016; Zanella et al, 2007; Zanella et al, 2005). These patients are considered "not regularly transfused." Some adult patients who have not been splenectomized require regular transfusions. A minority of adult patients with PK deficiency still require regular transfusions after splenectomy. There is no clear definition of what constitutes a regularly transfused patient with PK deficiency; data from the PK Deficiency NHS point to a wide range of transfusion frequencies in adults with the disease.

5.2. Investigational Product (AG-348)

5.2.1. Proposed Mechanism of Action of AG-348

AG-348 is a potent, broad-spectrum activator of PKR, 1 of 4 pyruvate kinase isoenzymes expressed in human tissues from 2 separate genes. Both PKR and the liver-specific form of pyruvate kinase (PKL) are splice isoforms of the *PKLR* gene, while pyruvate kinase muscle isozyme (PKM)1 and PKM2 are both expressed from the *PKM* gene. AG-348 is an allosteric activator of the PKR, PKL, and PKM2 isoenzymes, with similar activity for each. AG-348 acts by directly binding to the PKR tetramer and allosterically enhancing its affinity for PEP.

As described in Section 5.1.2, the activity of the glycolytic pathway is disrupted in patients with PK deficiency. This disruption results in significantly reduced RBC lifespan and manifests clinically as nonspherocytic hemolytic anemia. In patients with PK deficiency, RBCs and their progenitors are characterized by changes in metabolism associated with defective glycolysis, including a buildup of PEP and the intermediate 2,3-DPG, and lowered levels of ATP. It is hypothesized that AG-348 restores the ability of RBCs to convert PEP + ADP to pyruvate + ATP and thereby normalizes RBC metabolism in patients with PK deficiency.

5.2.2. Summary of AG-348 Nonclinical Data With Potential Clinical Interest

A series of exploratory pharmacology studies were conducted to characterize the ability of AG-348 to activate wild-type (WT) PKR and anemia-associated PKR mutants in vitro, ex vivo, and in vivo.

Biochemical studies showed that AG-348 is a potent, broad-spectrum activator of recombinant PKR with low nanomolar potency against both WT and mutant enzymes. The effect of AG-348 on PKR activity and a number of downstream pathway markers was evaluated in both human and murine RBCs and whole blood. AG-348 dose-response curves in human and murine RBCs showed increased PKR activity. AG-348 dose-response curves also showed increased ATP levels.

The effects of AG-348 on PKR activity and RBC metabolism were also 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-deficient patients with different mutations in the PKR enzyme. Finally, a series of 3 in vivo pharmacology studies conducted in C57BL/6 mice confirmed the in vitro potency of AG-348 in increasing WT PKR enzyme activity and in modulating the levels of the downstream markers, ATP, and 2,3-DPG. Based on the data from these studies, a strong pharmacokinetic/pharmacodynamic (PD) relationship was established between AG-348 area under the plasma concentration × time curve from 0 to 12 hours (AUC_{0-12}) and ATP/2,3-DPG AUC_{0-12} ratio.

AG-348 was evaluated for its potential to inhibit binding and enzymatic activity in a panel of 89 receptors, ion channels, and enzymes. AG-348 is a histamine H3 antagonist/inverse agonist and an aromatase inhibitor. Findings consistent with aromatase inhibition have been observed in the reproductive organs of male and female rats in toxicology studies of up to 6 months duration at exposures as low as 10,900 ng•hr/mL. No findings consistent with aromatase inhibition have been observed in monkeys in toxicology studies of up to 9 months duration. Effects consistent with aromatase inhibition and antagonism/inverse agonism at the H3 receptor have been observed in clinical studies (Section 5.2.3.3).

Emesis was observed in monkey toxicology studies, and dose-dependent emesis was observed in a dedicated safety pharmacology study in ferrets.

Based on animal studies, AG-348 may affect fertility in males and females. In animals, these effects were reversible after discontinuation of AG-348. AG-348 may also affect the ability to maintain pregnancy.

Further details on these and other nonclinical studies, including nonclinical pharmacokinetics, are in the AG-348 Investigator's Brochure (IB).

5.2.3. Summary of AG-348 Clinical Data

AG-348 has been evaluated in 4 clinical pharmacology studies in healthy subjects (3 completed and 1 ongoing) and 1 ongoing Phase 2, open-label, efficacy, and safety study (AG348-C-003, referred to as DRIVE-PK) in adult subjects with PK deficiency.

The ongoing DRIVE-PK study is an open-label study intended for adult subjects with PK deficiency who are considered transfusion independent (per protocol definition) with screening hemoglobin (Hb) concentration ≤12 g/dL or ≤11 g/dL for males and females, respectively. The study is designed to evaluate the safety, tolerability, and potential indicators of clinical activity of 2 dose levels of AG-348 (50 and 300 mg twice daily [BID]) administered for up to 24 weeks in the Core Period, and beyond 24 weeks in the Extension Period. The DRIVE-PK study is also intended to evaluate the pharmacokinetics of AG-348, the PD response (ATP and 2,3-DPG levels) after administration of AG-348, and additional PD biomarkers.

A brief overview of AG-348 pharmacokinetic and PD data is provided in Section 5.2.3.1 and Section 5.2.3.2, respectively. An overview of the available safety from these studies as well as preliminary efficacy results from the DRIVE-PK study is included in Section 5.2.3.3 and Section 5.2.3.4, respectively. Please refer to the AG-348 IB for additional details on all clinical studies and results.

5.2.3.1. Summary of AG-348 Pharmacokinetics

The pharmacokinetic profile of AG-348 has been well characterized in the Phase 1 single-ascending dose (SAD) study (AG348-C-001) and Phase 1 multiple-ascending dose (MAD) study (AG348-C-002), conducted in healthy adult subjects. The pharmacokinetics of AG-348 increased in a dose-proportional manner across tested doses in the SAD study and at lower doses in the MAD study. At higher dose levels in the MAD study, a less than dose-proportional increase was observed, attributed to the cytochrome P450 (CYP)3A4 induction effect of AG-348. The effective half-life of AG-348 has been estimated to be approximately 3 to 6 hours.

A capsule formulation was used in the SAD and MAD studies and the DRIVE-PK study. A tablet formulation has subsequently been introduced into DRIVE-PK and will also be used in this study. Prior to introducing the tablet formulation in clinical studies, a relative bioavailability study (AG348-C-005) was conducted in healthy subjects to compare the pharmacokinetics of the 2 formulations (ie, capsules and tablets). Systemic exposure to AG-348 appeared similar between formulations with an area under the plasma concentration × time curve (AUC) ratio of 1.05 and a maximum (peak) concentration (C_{max}) ratio of 1.19 for the tablet formulation compared with the capsule formulation. These results suggest that no dose adjustments are required with the tablet formulation. Therefore, when the tablet formulation is used, the same dose as that of the capsule can be used in ongoing and future clinical studies.

Additionally, in the ongoing Phase 2 DRIVE-PK study, conducted in adult subjects with PK deficiency, the pharmacokinetics of AG-348 in plasma have been evaluated. To date, pharmacokinetic data of AG-348 in adult subjects with PK deficiency were found to be similar to that observed in healthy adult subjects.

Please refer to the AG-348 IB for detailed information regarding the pharmacokinetics of AG-348.

5.2.3.2. Summary of AG-348 Pharmacodynamics

In the SAD and MAD studies, the concentration of 2,3-DPG decreased in a dose-dependent manner and returned to levels close to baseline by 72 hours following the final dose of AG-348. In the SAD study, after a single dose of AG-348, a minimal increase in the concentration of ATP was observed at 24 to 120 hours postdose. In contrast to the SAD study, significant increases in ATP were observed in the MAD study, and the concentration of ATP remained elevated through 120 hours after the final dose of AG-348.

In the DRIVE-PK study, conducted in adult subjects with PK deficiency, the PD responses of ATP and 2,3-DPG in whole blood have also been evaluated. In this study, no consistent pattern of decrease in concentration of 2,3-DPG or increase in ATP has been observed. The reason for this is not completely clear, but may be due in part to changes in reticulocyte concentrations in response to treatment with AG-348.

Refer to the AG-348 IB for detailed information regarding the PD properties of AG-348.

5.2.3.3. Summary of AG-348 Clinical Safety Data

Overall, AG-348 has been generally well tolerated among healthy adult subjects and adult subjects with PK deficiency. Important identified risks associated with administration of AG-348

in clinical studies include bone mineral density (BMD) decrease (including osteoporosis and osteopenia due to aromatase inhibition), withdrawal hemolysis, and insomnia (not clinically serious ie, not Grade 3 or Grade 4).

Potential risks of AG-348 administration include anaphylactoid reaction, aromatase inhibition, gastrointestinal disturbances, transaminase increases, and triglyceride increase. Transaminase increases are adverse events of special interest (AESIs) for AG-348. Please refer to Section 11.2.6 for additional information on AESIs and the AG-348 IB for a more detailed overview of available safety data.

5.2.3.4. Summary of AG-348 Efficacy Data

In the ongoing Phase 2 DRIVE-PK study in adult subjects with PK deficiency, the efficacy of AG-348 is primarily analyzed via evaluation of changes in Hb concentrations.

As of a data cutoff of 14 July 2017, a preliminary analysis indicates that of the 52 subjects who received AG-348 during the Core Period, 26 subjects (50.0%) achieved maximum increases in Hb >1 g/dL (Grace et al, 2017). Of the 42 subjects with ≥1 missense mutation, 25 subjects (59.5%) had an Hb increase >1.0 g/dL. The majority of Hb increases were rapid and sustained. The median time to the first observation of an Hb increase >1 g/dL above baseline was 10 days (range 7 to 187 days).

Overall, treatment with AG-348 has resulted in Hb responses that are rapid in onset, robust, and sustained with prolonged treatment with AG-348. In summary, this clinical evidence from Study AG348-C-003 suggests that treatment with AG-348 has the ability to provide a significant clinical benefit to patients.

5.3. Study Rationale

The totality of the preliminary data from the Phase 2 DRIVE-PK study, combined with the data from the 2 completed Phase 1 SAD and MAD studies, support continued clinical development of AG-348 for the treatment of patients with PK deficiency. In order to evaluate the efficacy and safety of AG-348 across the disease spectrum of PK deficiency, 2 studies investigating the treatment of AG-348 in subjects with PK deficiency will be conducted. This protocol, AG348-C-006, will be conducted in adult subjects with PK deficiency who are not regularly receiving transfusions, while a separate study, AG348-C-007, will be conducted in adult subjects with PK deficiency who are regularly receiving transfusions.

A clear and serious unmet medical need exists for patients with PK deficiency. At present, there are no approved, disease-specific therapeutic agents for the treatment of patients with PK deficiency; rather, available treatment options are supportive only. Many adult patients underwent splenectomy in childhood as a means to reduce hemolysis and eliminate the need for regular transfusions. However, some patients, despite undergoing splenectomy, still have Hb concentration below the lower limit of normal and have symptoms associated with anemia, such as shortness of breath, fatigue, lower energy, lower exercise tolerance, and other symptoms that affect quality of life (QoL).

Treatment with AG-348 has the potential to correct the underlying pathology of PK deficiency by activating PKR and increasing glycolytic pathway activity in RBCs to reduce hemolysis and provide clinical benefit to subjects with PK deficiency.

5.3.1. Justification of Study Design

This is a 2-part, Phase 3, randomized, multicenter, double-blind, placebo-controlled study in which subjects will be randomized 1:1 to receive either AG-348 or matching placebo. The randomized controlled trial design was chosen as the most robust design for the evaluation of safety and efficacy of AG-348, with the best potential for minimizing bias.

The randomization will be stratified by the average of screening Hb concentrations (<8.5 vs ≥8.5 g/dL [5.28 mmol/L]) and the *PKLR* gene mutation category (missense/missense vs missense/non-missense). These stratification factors were chosen because both the average of screening Hb concentrations and the *PKLR* gene mutation category are related to the Hb response based on preliminary data from the Phase 2 DRIVE-PK study.

In Part 1 (Dose Optimization Period), subjects will follow a dose titration schema (as described in Section 5.3.2.1) to identify their individually optimized dose of study treatment (ie, AG-348/matching placebo). In Part 2 (Fixed Dose Period), subjects will continue treatment with their individually optimized dose. Subjects will be treated in this study for 24 weeks (not including the recommended dose taper), including 12 weeks for the Dose Optimization Period and 12 weeks on the Fixed Dose Period. Additional details on the study design are provided in Section 7.1. See Section 5.3.1.1, Section 5.3.1.2, and Section 5.3.2 for further justification regarding specific aspects of the study design.

5.3.1.1. Justification of Subject Population

This study will enroll adult subjects with PK deficiency and moderate to severe anemia (Hb ≤10 g/dL [6.21 mmol/L]), who are not regularly receiving transfusions. For the purpose of this study, the following definition will be used for subjects who are not regularly receiving transfusions: no more than 4 transfusion episodes in the 12-month period up to the first day of study treatment **and** no transfusions in the 3 months prior to the first day of study treatment.

As mentioned in Section 5.1.3, there are no published guidelines on the use of transfusion, and the appropriate transfusion frequency in PK deficient patients who may need transfusions. Hemolysis in patients with PK deficiency can be acutely exacerbated by infection, surgery, pregnancy, and other forms of stress. Since several such episodes can occur per year, it is considered that up to 4 transfusion episodes in the preceding 12 months reflects such stressors rather than a true dependency on regular transfusions. Because the Hb concentration, which informs the primary endpoint of the study, is influenced by transfusions in the preceding several months, the most recent transfusion must have occurred at least 3 months prior to the first day of study treatment.

Additional inclusion criteria for this study have been added to increase the likelihood that the subject population will achieve a clinical benefit from the study treatment. This study will require an average screening Hb concentration of ≤10 g/dL (6.21 mmol/L), to ensure that all subjects enrolled in the study have a moderate-to-severe degree of anemia.

Subjects who are homozygous for the R479H mutation or have 2 non-missense mutations, without the presence of another missense mutation, in the *PKLR* gene will not be included in this study. It is expected that non-missense mutations are more likely to result in loss of functional protein expression, as opposed to the missense mutations, which result in a single amino acid substitution. Genotype-response analysis in the DRIVE-PK study demonstrated in general that

all 10 PK-deficient subjects with 2 non-missense alleles did not respond to treatment with AG-348. Additionally, all 5 subjects with the R479H/R479H genotype (drawn from the [redacted]) in the DRIVE-PK study were non-responders.

5.3.1.2. Justification of Study Objectives

The primary endpoint of this study will be the assessment of the Hb response (HR). Additionally, Hb concentrations over time and maximal Hb changes will be assessed as secondary efficacy measures.

Hemoglobin response will be defined as a ≥1.5 g/dL (0.93 mmol/L) increase in Hb concentration from baseline that is sustained at 2 or more scheduled assessments at Weeks 16, 20, and 24 during the Fixed Dose Period. A sustained Hb increase of ≥1.5 g/dL represents a clinically meaningful benefit as suggested by the standard use of splenectomy and transfusions for patients with PK deficiency. Splenectomy increases Hb by 1-3 g/dL and is considered an effective and standard treatment in PK deficiency (Grace et al, 2015; Zanella et al, 2007; Zanella et al, 2005), while transfusions, typically of 2 units of packed RBCs, increase Hb by about 2 g/dL in adults (Weinstein, 2012). There is abundant literature suggesting that such an increase in Hb delivers an immediate subjective benefit in patients (Ryblom et al, 2015). In particular, the FACT-An (Functional Assessment of Cancer Therapy Anemia) instrument has shown that transfusions result in an Hb increase that is above the value defined as a minimal clinically important difference (MCID) (Cella et al, 2002). Importantly, a substantial positive correlation between post-transfusion Hb concentrations and FACT-An scores has been established.

Pyruvate kinase deficiency is a chronic hemolytic anemia. The hemolysis itself leads to morbidity (eg, jaundice, gall stones). Another important adverse consequence of the disease is the risk of iron overload, worsened by frequent transfusions and splenectomy. Altogether, these factors negatively impact patients' QoL. Therefore, this study is designed to investigate multiple additional objective and subjective measures in PK deficiency, among them hematopoietic activity, hemolysis, iron overload, and QoL.

5.3.2. Rationale for Dose

To assist with dose selection, an exposure-response analysis was conducted using pharmacokinetic, efficacy, and safety data from the DRIVE-PK study.

Briefly, a sequential population pharmacokinetic-efficacy model was developed using increase in Hb as the efficacy endpoint, while a binary logistic regression approach incorporating the safety endpoints of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and free testosterone, estrone, estradiol, insomnia, and hot flush was used for the analysis of exposure-safety relationship. Insomnia was the only safety event that was found to be significant in the logistic regression analysis.

Following model development, simulations were conducted to select 3 dose levels for evaluation in Phase 3 studies:

1. The low dose level was identified as a dose at which patients were likely to have an Hb increase of ≥1.5 g/dL (0.93 mmol/L) from baseline without exceeding the upper limit of normal (ULN) Hb (efficacy criterion), with a minimal probability of occurrence of Grade ≥1 insomnia (safety criterion).

2. Since the mid dose (20 mg BID) involves an intra-patient dose level increase, this dose level was selected such that it would result in a reasonable increase (approximately 2- to 2.5-fold) in exposure compared to the predicted exposure at the low dose level.
3. Since the high dose (50 mg BID) also involves an intra-patient dose level increase, this dose level was selected such that it would result in a reasonable increase (approximately 2- to 2.5-fold) in exposure compared to the predicted exposure at the mid dose level.

Using these criteria and simulations from the population pharmacokinetic-efficacy-safety analyses, doses of 5 mg BID, 20 mg BID, and 50 mg BID were selected for the Dose Optimization Period.

5.3.2.1. Justification of Individual Dose Optimization

In the DRIVE-PK study of AG-348 in PK deficiency, subjects were randomized to receive doses of either 50 mg or 300 mg BID of AG-348. The preliminary results of this study indicated that efficacy could be achieved at lower doses than 300 mg in the majority of subjects. More specifically, several subjects had to have their randomized dose level reduced because of excess increases in Hb or the occurrence of adverse events (AEs), such as insomnia, headache, or nausea. Dose reductions led to the resolution of most of these AEs and the maintenance of a satisfactory but not excessive level of Hb. In the pivotal studies, Hb overshoots will be avoided by gradually titrating the dose upward in each subject.

For this reason, all subjects will start on an initial dose of study treatment of 5 mg BID with 2 potential sequential steps for dose level increases (ie, from 5 to 20 mg BID and from 20 to 50 mg BID), depending on safety and Hb change.

Preliminary data from the DRIVE-PK study indicated that subjects with PK deficiency who respond to activation of the PKR protein typically do so within 2-3 weeks. Therefore, every 4 weeks during Part 1, subjects will be assessed for safety and efficacy (as defined by Hb increase) to determine if their dose should be increased, maintained at the current level, or decreased.

Individual dose optimization is incorporated in this study to allow each subject to gradually increase his/her dose of AG-348 in order to identify a dose that confers maximum benefit with minimum risk to that subject. This approach is made possible by the rapid responses to activation of PKR that were observed in the DRIVE-PK study and that are consistent with the mechanism of action of this class of drugs.

Details on maintaining the study blind during the dose-optimization part of the study can be found in Section 9.3.

6. TRIAL OBJECTIVES AND ENDPOINTS

6.1. Objectives

6.1.1. Primary Objective

The primary objective of the study is to evaluate the efficacy of treatment with AG-348 compared with placebo in increasing Hb concentrations.

6.1.2. Secondary Objectives

- To evaluate the safety of AG-348
- To determine the effect of the study treatment regimens on markers of hemolysis, hematopoietic activity, and other indicators of clinical activity
- To determine the effect of the study treatment regimens on health-related quality of life (HRQoL), as determined using patient-reported outcomes (PROs)
- To evaluate the pharmacokinetics of AG-348 after oral administration
- To evaluate the relationship between AG-348 pharmacokinetics and safety parameters

6.1.3. Exploratory Objectives

- To evaluate the relationship of AG-348 pharmacokinetics to indicators of clinical activity
- To evaluate the PD markers of PK deficiency and how they are affected by study treatment
- To determine the effect of the study treatment regimens on:
  - Number of transfusion events and number of RBC units transfused
  - Markers of iron metabolism and indicators of iron overload

6.2. Endpoints

6.2.1. Primary Endpoint

The primary endpoint is the HR, defined as a ≥1.5 g/dL (0.93 mmol/L) increase in Hb concentration from baseline that is sustained at 2 or more scheduled assessments at Weeks 16, 20, and 24 during the Fixed Dose Period. The individual subject's baseline Hb concentration is defined as the average of all available Hb concentrations collected for that subject during the Screening Period up to the first dose of study treatment.

6.2.2. Key Secondary Endpoint

The key secondary endpoint is the average change from baseline in Hb concentration at Weeks 16, 20, and 24.

6.2.3. Other Secondary Endpoints

- Maximal Hb concentration increase from baseline
- Time to first achieve an increase in Hb concentration of 1.5 g/dL (0.93 mmol/L) or more from baseline
- Average change from baseline at Weeks 16, 20, and 24 in markers of hemolysis: bilirubin, lactate dehydrogenase (LDH), and haptoglobin levels
- Average change from baseline at Weeks 16, 20, and 24 in markers of hematopoietic activity: reticulocyte percentages
- Change from baseline in HRQoL PRO scores: Pyruvate Kinase Deficiency Diary (PKDD) and Pyruvate Kinase Deficiency Impact Assessment (PKDIA)
- Safety endpoints, including: the type, incidence, severity, and relationship to study treatment of AEs and serious adverse events (SAEs); number of discontinuations due to AEs; results of clinical laboratory tests over time (eg, serum chemistry, liver function tests (LFTs), hematology, lipids, sex steroids, urinalysis, coagulation); physical examination (PE) findings; dual-energy x-ray absorption (DXA) scans; vital signs; 12-lead electrocardiogram (ECG) data
- Pharmacokinetic endpoints, including plasma concentrations over time and pharmacokinetic parameters of AG-348 (eg, AUC, C_{max}, others as applicable)
- Exposure-response relationship between safety parameters and AG-348 concentration and relevant AG-348 pharmacokinetic parameters

6.2.4. Exploratory Endpoints

- Exposure-response (or pharmacokinetic-pharmacodynamic) relationship between relevant pharmacokinetic parameters and endpoints that are indicators of clinical activity
- Change from baseline in additional markers of hematopoietic activity
- Change from baseline in markers of iron metabolism and indicators of iron overload
- Change from baseline in PKR protein level
- Relationship between baseline PKR protein level and Hb response status
- Change from baseline in HRQoL PRO scores: European quality of life five-dimensional descriptive system (EQ-5D-5L)
- Change from baseline in PKR flux assay results
- Proportion of subjects requiring transfusions and the total number of RBC units transfused

7. INVESTIGATIONAL PLAN

7.1. Study Design

7.1.1. Overview of Study Design

This is a Phase 3, randomized, multicenter, double-blind, placebo-controlled study consisting of a Dose Optimization Period (Part 1) followed by a Fixed Dose Period (Part 2) as shown in Figure 2. This study will evaluate the efficacy and safety of orally administered AG-348 as compared with placebo in subjects with PK deficiency who are not regularly receiving blood transfusions. Blinding and unblinding procedures are detailed in Section 9.3 and Section 9.4, respectively.

Approximately 76 subjects will be randomized 1:1 to receive either AG-348 or matching placebo. The term "study treatment" is used throughout the protocol to define both the active (AG-348) drug and placebo. Randomization will be stratified by the average of screening Hb concentrations (<8.5 vs ≥8.5 g/dL [5.28 mmol/L]) and the *PKLR* gene mutation category (missense/missense vs missense/non-missense). In rare instances where *PKLR* gene mutation category cannot be made definitively (eg, if a subject harbors 3 mutant *PKLR* alleles), the subject will be assigned to the missense/non-missense category.

Each subject's course of participation will be composed of the following periods:

Screening Period: Prior to randomization, there will be a Screening Period of up to 42 days in which a subject's eligibility for the study will be determined and confirmed. A subject's Screening Period duration may be extended beyond 42 days upon the Medical Monitor's, or designee's, approval.

Dose Optimization Period (Part 1): Following randomization, subjects will enter Part 1 of the study. During Part 1, subjects will receive scheduled dose increases depending on their response to study treatment (safety and efficacy), as detailed in Section 7.1.3, to determine their individually optimized dose level.

Fixed Dose Period (Part 2): Following Part 1, subjects will proceed to Part 2 and continue study treatment on their individually optimized dose. For the purposes of dosing during the Fixed Dose Period, the dose the subject is being administered at the Week 12 Visit will be considered the subject's optimized dose and will be the dose the subject receives during Part 2. All subjects who remain on study during Part 2 through the Week 24 Visit may be eligible for an open-label extension study, in which all subjects will receive AG-348.

Discontinuation and follow-up: Subjects who discontinue the study prior to the Week 24 Visit should attend the End of Study Visit 28±4 days after the last study visit that the subject attended, or 28±4 days after the last dose of study treatment (including the recommended dose taper), whichever is later. The End of Study Visit will be identical to the Week 24 Visit with these exceptions: no study treatment will be dispensed, and if the subject has not already done so, the subject should return their electronic diary (eDiary) and remaining study treatment. These subjects do not need to complete the Follow-Up visit.

Subjects who continue the study through the Week 24 Visit on study treatment but do not continue on into an extension study, will attend the Follow-up Visit 28±4 days after the last dose

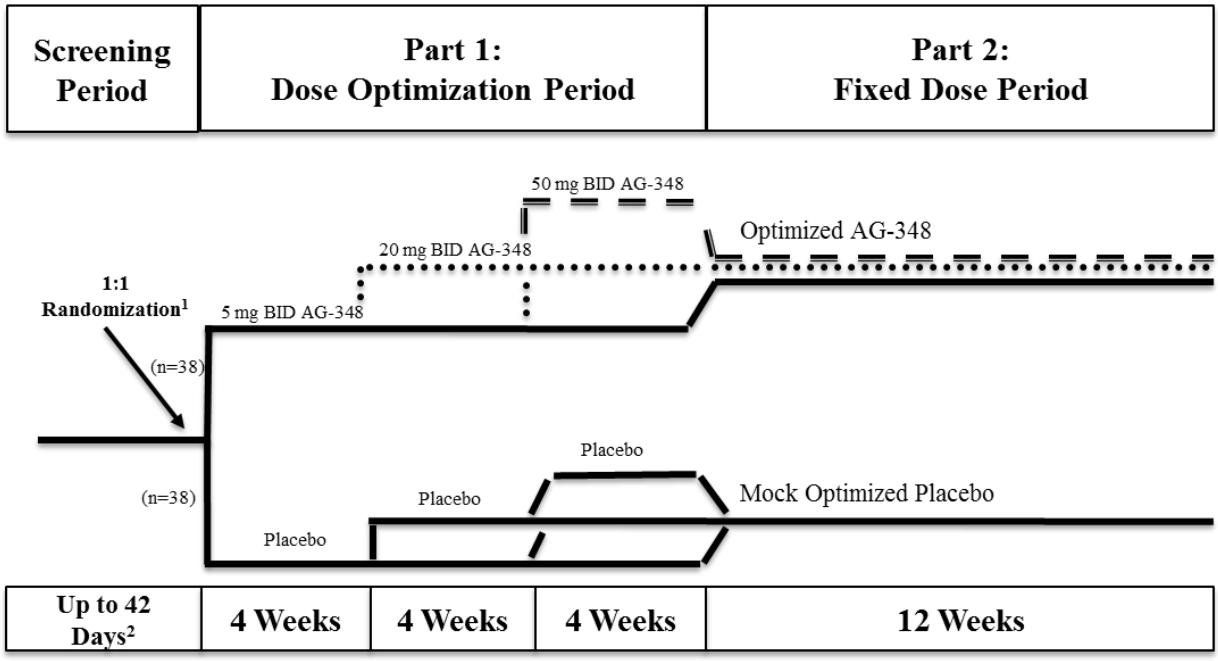
of study treatment. Subjects who continue on into an extension study at the Week 24 Visit will not be required to attend the Follow-up Visit.

All subjects who discontinue or interrupt study treatment during the study should undergo the recommended dose taper (see Section 9.5.3), unless an emergency situation justifies discontinuing or interrupting the study treatment abruptly. Whether or not the recommended dose taper is performed, subjects who discontinue or interrupt study treatment should be monitored as clinically indicated for signs of withdrawal hemolysis and worsening of anemia.

The maximum total duration that a subject can receive study treatment in this study is 24 weeks (not including the recommended dose taper).

The Screening Period, Part 1, Part 2, and follow-up are described in more detail in Sections 7.1.2, 7.1.3, 7.1.4, and 7.1.5, respectively.

Figure 2: Overview of Design for Study AG348-C-006



Abbreviations: BID = twice daily.
[1]Stratified by average of screening Hb concentrations (Hb <8.5 g/dL vs Hb ≥8.5 g/dL [5.28 mmol/L]) and the *PKLR* gene mutation category (missense/missense vs missense/non-missense)
[2] A subject's Screening Period duration may be extended beyond 42 days upon the Medical Monitor's, or designee's, approval.

7.1.2. Screening Period

Following signing of the informed consent form (ICF), subjects will enter the Screening Period to determine eligibility. Screening assessments will be performed after the signing of the ICF and within 42 days prior to Day 1 of the study. A subject's Screening Period duration may be extended beyond 42 days upon the Medical Monitor's, or designee's, approval.

The Investigator will determine whether each subject meets all the inclusion criteria and none of the exclusion criteria (see Section 8). Eligibility of each subject will be confirmed by the Medical

Monitor, or designee. A subject cannot be randomized (and cannot receive his/her first dose of study treatment) until eligibility is confirmed.

Subjects will receive an eDiary at screening to document dosing, HRQoL assessments, and menstrual cycle (for menstruating women) throughout the study.

For each subject, the following information will be collected during screening (see Section 10.13):

- Pyruvate kinase deficiency specific assessments: PKR enzyme assay, *PKR* genotyping, and dates of all transfusions received during the 12-month period prior to the first day of study treatment
- General assessments: Medical/surgical history (as detailed in Section 10.4), demographics, prior medications, PE, 12-lead ECG, vital signs, hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) antibody (Ab), human immunodeficiency virus (HIV)-1 and -2 Ab, and follicle-stimulating hormone (FSH) (to confirm post-menopausal status, as applicable) or serum human chorionic gonadotropin (hCG) pregnancy test (in women of reproductive potential)
- Patient-reported outcome assessments: PKDD daily and PKDIA, FACT-An, SF12v2, EQ-5D-5L, and PGIS all on the first day of screening (+1 day) and then at weekly intervals (±1 day) until Part 1 Day 1
- Assessment of iron overload: Liver magnetic resonance imaging (MRI) to assess liver iron concentration (LIC); iron panel and related markers
- Other: DXA scans
- Safety and efficacy laboratory markers: Hematology; LDH and haptoglobin
  - At least 2 hematology labs will be collected (at least 7 days apart)
- Safety laboratory markers: Serum chemistry, LFTs, coagulation studies, urinalysis, and lipids
- Other laboratory markers: Markers of erythropoietic activity

7.1.3. Part 1: Dose Optimization Period

On Day 1, the following additional conditions must be met before a subject, who is considered eligible after completing screening, can receive the first dose of study treatment:

- The subject must not have received a transfusion since completing screening (see Section 8.1, Inclusion Criterion 5).
- Women of reproductive potential must have a negative urine or serum pregnancy test (see the Schedule of Assessments Section 10.13).

Following randomization to either AG-348 or matching placebo, subjects will enter the Dose Optimization Period (Part 1), a 12-week period starting on Day 1 of the study. The goal of the Dose Optimization Period is to maximize a subject's increase in Hb while maintaining an acceptable safety profile. All subjects will receive an initial dose of 5 mg BID of study treatment with 2 potential sequential steps for dose level increase (ie, from 5 to 20 mg BID and from 20 to

50 mg BID; no increases beyond 50 mg BID will be allowed). The first dose of study treatment on Day 1 should be taken at the study site following all Day 1 assessments (with the exception of eDiary assessments) as depicted in the Schedule of Assessments (Section 10.13).

Subjects will be assessed for safety and efficacy (as defined by Hb increase) every 4 weeks during Part 1, to determine if their dose should be increased, maintained at the current level, or decreased. At the Week 4 and Week 8 Visits, study treatment dose should be increased to the next dose level if the subject has met both of the following criteria:

- The subject is tolerating the study treatment, **and**
- The subject's Hb concentration on the day of the visit based on local laboratory results is lower than 2.5 g/dL (1.55 mmol/L) below the upper limit of normal (ULN), as applies to men and women.

Dose re-escalation or re-introduction should be avoided after the Week 8 Visit, but may be permitted, after discussion with the Independent Medical Monitor, or designee.

At the Week 12 Visit, if the subject has tolerated the study treatment, the subject will remain at his/her current dose level. If the Investigator deems it necessary to reduce the study treatment for safety reasons, the subject's dose may be reduced to 1 of the 2 available lower dose levels (ie, 5 mg BID, 20 mg BID). If the subject is already receiving 5 mg BID and/or cannot tolerate BID dosing, another regimen may be allowed after discussion with, and approval by, the Independent Medical Monitor, or designee.

If questions arise about whether the dose level of a given subject should be increased, maintained, or decreased, the Independent Medical Monitor, or designee, should be contacted.

At any time during the study, the Investigator can discontinue, interrupt, or reduce the subject's dose of study treatment for reasons related to safety (as described in Section 9.5).

Over the course of Part 1 of the study, subjects will attend visits on Day 1, Week 2, Week 4, Week 6, Week 8, Week 10, and Week 12. Details on the timing and type of assessments for Part 1 are outlined in the Schedule of Assessments (Section 10.13).

7.1.4. Part 2: Fixed Dose Period

Part 2 is the 12-week period following the Week 12 Visit through Week 24.

Following Part 1, each subject will remain on his/her individually optimized dose and enter the Fixed Dose Period (Part 2). For the purposes of dosing during the Fixed Dose Period, the dose the subject is being administered at the Week 12 Visit will be considered the subject's optimized dose and will be the dose the subject receives during Part 2.

At any time during the study, the Investigator can discontinue, interrupt, or reduce the subject's dose of study treatment for reasons related to safety (as described in Section 9.5).

Dose re-escalation or re-introduction should be avoided after the Week 8 Visit (Part 1), but may be permitted, after discussion with the Independent Medical Monitor, or designee.

Subjects will visit the study site every 4 weeks through Week 24 (ie, Week 16, Week 20, and Week 24). Each study visit will include efficacy and safety assessments. Assessments will be performed as outlined in the Schedule of Assessments (Section 10.13).

7.1.5. Study Discontinuation and Follow-up

Subjects who discontinue the study prior to the Week 24 Visit should attend the End of Study Visit 28±4 days after the last study visit that the subject attended or 28±4 days after the last dose of study treatment (including the recommended dose taper), whichever is later. This visit will be identical to the Week 24 Visit with these exceptions: no study treatment will be dispensed at the End of Study Visit, and if the subject has not already done so, they should return their eDiary and remaining study treatment. These subjects do not need to complete the Follow-Up Visit.

Subjects who continue study treatment through Week 24 should continue taking study treatment at least through the morning dose of the Week 24 Visit. All subjects who remain on study during Part 2 through the Week 24 Visit may be eligible for an open-label extension study, in which all subjects will receive AG-348.

- Subjects who continue study treatment through the Week 24 Visit but do not continue on into an extension study, should undergo the recommended dose taper and then attend the Follow-up Visit 28±4 days after the last dose of study treatment (including the recommended dose taper).
- Subjects who continue on into an extension study at the Week 24 Visit will not be required to attend the Follow-up Visit.
- Subjects who initiate or are undergoing the recommended dose taper, and for whom participation in an extension study remains undetermined, should perform the taper as detailed in Section 9.5.3. Transition to an extension study should be discussed with the Independent Medical Monitor.

All subjects who discontinue or interrupt study treatment during the study should undergo the recommended dose taper (see Section 9.5.3), unless an emergency situation justifies discontinuing or interrupting the study treatment abruptly.

Guidelines for the follow-up of subjects with any AE at the subject's completion of the study are described in Section 11.

7.2. Number of Subjects

Approximately 76 subjects are planned for enrollment.

7.3. 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 the following:

- Determination of unexpected, significant, or unacceptable risk to subjects (eg, as determined by the Independent Data Monitoring Committee [IDMC]).
- Plans to modify, suspend, or discontinue the development of the study treatment.
- Decisions of competent authorities or Institutional Review Board (IRB)/Independent Ethics Committee (IEC)

- Other administrative reasons.

Should the study be closed prematurely, all study materials must be returned to the Sponsor or the Sponsor's designee.

8. SELECTION AND WITHDRAWAL OF SUBJECTS

8.1. Inclusion Criteria

For enrollment into this study, subjects must meet all of the following criteria during the Screening Period:

1. Have provided signed written informed consent prior to performing any study procedure, including screening procedures.
2. Be aged 18 years or older.
3. Have documented clinical laboratory confirmation of PK deficiency, defined as documented presence of at least 2 mutant alleles in the *PKLR* gene, of which at least 1 is a missense mutation, as determined per the genotyping performed by the central genotyping laboratory.
4. Have an Hb concentration less than or equal to 10.0 g/dL (6.21 mmol/L) regardless of gender (average of at least 2 Hb measurements [separated by a minimum of 7 days] during the Screening Period).
5. Be considered not regularly transfused, defined as having had no more than 4 transfusion episodes in the 12-month period up to the first day of study treatment **and** no transfusions in the 3 months prior to the first day of study treatment.
6. Have received at least 0.8 mg oral folic acid daily for at least 21 days prior to the first dose of study treatment, to be continued daily during study participation.
7. Have adequate organ function, as defined by:
    a. Serum AST $\leq$2.5 × ULN (unless the increased AST is assessed by the Investigator as due to hemolysis and/or hepatic iron deposition) and ALT $\leq$2.5 × ULN (unless the increased ALT is assessed by the Investigator as due to hepatic iron deposition).
    b. Normal or elevated levels of serum bilirubin. In subjects with serum bilirubin >ULN, the elevation must not be associated with choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease. Elevated bilirubin attributed to hemolysis with or without Gilbert's syndrome is not exclusionary.
    c. Estimated glomerular filtration rate (GFR) $\geq$60 mL/min/1.73 m^2, measured GFR $\geq$60 mL/min, or calculated creatinine clearance (CrCL; Cockcroft-Gault) $\geq$60 mL/min.
    d. Absolute neutrophil count (ANC) $\geq 1.0 \times 10^9$/L (based on an average of at least 2 measurements [separated by a minimum of 7 days] during the Screening Period).
    e. Platelet count $\geq 100 \times 10^9$/L in the absence of a spleen, or platelet count $\geq 50 \times 10^9$/L in the presence of a spleen and in the absence of any other cause of thrombocytopenia (based on an average of at least 2 measurements [separated by a minimum of 7 days] during the Screening Period).
    f. Activated partial thromboplastin time (aPTT) and international normalized ratio (INR) $\leq$1.25 × ULN, unless the subject is receiving therapeutic anticoagulants.
8. For women of reproductive potential, have a negative serum pregnancy test during the Screening Period. Women of reproductive potential are defined as sexually mature women who have not undergone a hysterectomy, bilateral oophorectomy, or tubal

occlusion; or who have not been naturally postmenopausal (ie, who have not menstruated at all for at least the preceding 12 months prior to signing informed consent and have an elevated FSH level indicative of menopause during the Screening Period).

9. For women of reproductive potential as well as men with partners who are women of reproductive potential, be abstinent as part of their usual lifestyle, or agree to use 2 forms of contraception, 1 of which must be considered highly effective, from the time of giving informed consent, during the study, and for 28 days following the last dose of study treatment for women and 90 days following the last dose of study treatment for men. A highly effective form of contraception is defined as combined (estrogen and progestin containing) hormonal contraceptives (oral, intravaginal, or transdermal) known to be associated with inhibition of ovulation; progestin-only hormonal contraceptives (oral, injectable, or implantable) known to be associated with inhibition of ovulation; intrauterine device; intrauterine hormone releasing system; bilateral tube occlusion; or vasectomized partner. The second form of contraception can include an acceptable barrier method, which includes male or female condoms with or without spermicide, and cervical cap, diaphragm, or sponge with spermicide. Women of reproductive potential using hormonal contraception as a highly effective form of contraception must also utilize an acceptable barrier method while enrolled in the study and for at least 28 days after their last dose of study treatment.
10. Be willing to comply with all study procedures for the duration of the study.

8.2. Exclusion Criteria

Subjects who meet any of the following criteria during screening will not be enrolled in the study:

1. Are homozygous for the R479H mutation or have 2 non-missense mutations, without the presence of another missense mutation, in the *PKLR* gene as determined per the genotyping performed by the central genotyping laboratory.
2. Have a significant medical condition that confers an unacceptable risk to participating in the study, and/or that could confound the interpretation of the study data. Such significant medical conditions include, but are not limited to the following:
    a. Poorly controlled hypertension (defined as systolic blood pressure [BP] >150 mmHg or diastolic BP >90 mmHg) refractory to medical management.
    b. History of recent (within 6 months prior to signing informed consent) congestive heart failure; myocardial infarction or unstable angina pectoris; hemorrhagic, embolic, or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.
    c. Cardiac dysrhythmias judged as clinically significant by the Investigator.
    d. Heart-rate corrected QT interval-Fridericia's method (QTcF) >450 msec (average of triplicate ECGs) with the exception of subjects with right or left bundle branch block.
    e. Clinically symptomatic cholelithiasis or cholecystitis. Prior cholecystectomy is not exclusionary. Subjects with symptomatic cholelithiasis or cholecystitis may be rescreened once the disorder has been treated and clinical symptoms have resolved.
    f. History of drug-induced cholestatic hepatitis.

g. Iron overload sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac (eg, clinically significant impaired left ventricular ejection fraction), hepatic (eg, fibrosis, cirrhosis), or pancreatic (eg, diabetes) dysfunction.
h. Have a diagnosis of any other congenital or acquired blood disorder or any other hemolytic process, except mild allo-immunization, as a consequence of transfusion therapy. Genetic findings that in isolation are predicted to be insufficient to explain the observed clinical phenotype may be allowed (eg, heterozygous status for certain recessive red blood cell disorders).
i. Positive test for HBsAg or HCVAb with signs of active hepatitis B or C virus infection. If the subject is positive for HCVAb, a reverse transcriptase-polymerase chain reaction test will be conducted. Subjects with hepatitis C may be rescreened after receiving appropriate hepatitis C treatment.
j. Positive test for HIV-1 or -2 Ab.
k. Active infection requiring the use of parenteral antimicrobial agents or Grade ≥3 in severity (per NCI CTCAE [National Cancer Institute Common Terminology Criteria for Adverse Events) within 2 months prior to the first dose of study treatment.
l. Diabetes mellitus judged to be under poor control by the Investigator or requiring >3 antidiabetic agents, including insulin (all insulins are considered 1 agent); use of insulin per se is not exclusionary.
m. History of any primary malignancy, with the exception of: curatively treated nonmelanomatous skin cancer; curatively treated cervical or breast carcinoma in situ; or other primary tumor treated with curative intent, no known active disease present, and no treatment administered during the last 3 years.
n. Unstable extramedullary hematopoiesis that could pose a risk of imminent neurologic compromise.
o. Current or recent history of psychiatric disorder that, in the opinion of the Investigator or Medical Monitor, or designee, could compromise the ability of the subject to cooperate with study visits and procedures.

3. Have a splenectomy scheduled during the study treatment period or have undergone splenectomy within 12 months prior to signing informed consent.
4. Are currently enrolled in another therapeutic clinical trial involving ongoing therapy with any investigational or marketed product or placebo. Prior and subsequent participation in the PK Deficiency NHS (NCT02053480) or PK Deficiency Registry is permitted, however, concurrent participation is not. Therefore, subjects enrolling in this current study will be expected to temporarily suspend participation in the NHS or Registry.
5. Have exposure to any investigational drug, device, or procedure within 3 months prior to the first dose of study treatment.
6. Have had any prior treatment with a pyruvate kinase activator.
7. Have a prior bone marrow or stem cell transplant.
8. Are currently pregnant or breastfeeding.
9. Have a history of major surgery within 6 months of signing informed consent. Note that procedures such as laparoscopic gallbladder surgery are not considered major in this context.

10. Are currently receiving medications that are strong inhibitors of CYP3A4, strong inducers of CYP3A4, strong inhibitors of P-glycoprotein (P-gp), or digoxin (a P-gp sensitive substrate medication) that have not been stopped for a duration of at least 5 days or a timeframe equivalent to 5 half-lives (whichever is longer) prior to the first dose of study treatment.
11. Are currently receiving hematopoietic stimulating agents (eg, erythropoietins, granulocyte colony stimulating factors, thrombopoietins) that have not been stopped for a duration of at least 28 days prior to the first dose of study treatment.
12. Have a history of allergy to sulfonamides if characterized by acute hemolytic anemia, drug-induced liver injury, anaphylaxis, rash of erythema multiforme type or Stevens-Johnson syndrome, cholestatic hepatitis, or other serious clinical manifestations.
13. Have a history of allergy to AG-348 or its excipients (microcrystalline cellulose, croscarmellose sodium, sodium stearyl fumarate, and mannitol).
14. Are currently receiving anabolic steroids, including testosterone preparations, within 28 days prior to the first dose of study treatment.

8.3. Subject Withdrawal Criteria

Subjects may withdraw from the study at any time for any reason. Subjects will be withdrawn from treatment and study-related procedures under the following conditions:

- Withdrawal of consent
- Development of an intercurrent medical condition that precludes further participation in the study
- Subject requires use of a prohibited concomitant medication (Section 9.7.1)
- Investigator decision
- Persistent nonadherence to protocol requirements
- Pregnancy
- Lost to follow-up

A subject who discontinues treatment will be encouraged to return for subsequent scheduled visits (or at a minimum the End of Study Visit) unless the subject withdraws consent. Should a subject decide to withdraw consent, 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 treatment or the study, the Independent Medical Monitor, or designee, must be informed.

When a subject withdraws from the study treatment or withdraws from the study, the primary reason for treatment discontinuation or study discontinuation must be recorded in the appropriate section of the electronic case report form (eCRF).

Refer to Section 11 for details regarding the follow-up of AEs ongoing at the time a subject discontinues treatment.

8.4. Subject Replacement

Subjects will not be replaced.

8.5. End of Study

End of Study is defined as the time at which all subjects have completed the study or are lost to follow-up.

9. TREATMENT OF SUBJECTS

9.1. Description of Study Treatment

9.1.1. Study Treatment

The term "study treatment" is used throughout this protocol to define both the active (AG-348) drug and placebo. AG-348 will be supplied as 5, 20, and 50 mg strength tablets to be administered orally. Placebo will be supplied as matching tablets to be administered orally.

Please see the IB for further details regarding AG-348. AG-348 is provided for investigational use only (considered an investigational medicinal product [IMP]) and is to be used only within the context of this study. All study treatment product will be supplied by the Sponsor.

9.1.2. Study Treatment Packaging and Labeling

AG-348 and matching placebo will be supplied as 7-day blister wallets and will be labeled appropriately as IMP for this study. The labeling for AG-348 and matching placebo will be identical.

Subjects will be receiving 1 of 3 potential doses, each of which is supplied as a different sized tablet. Thus, to maintain blinding, each dose of study treatment will be supplied as 3 different sized tablets: 1 tablet will be the active drug and the other 2 tablets will be placebo for subjects who are randomized to active, and all 3 tablets will be placebo for subjects who are randomized to placebo. The packaging will not change if the subject undergoes the recommended dose taper.

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

9.1.3. Study Treatment Storage

AG-348 and matching placebo tablets must be stored according to the respective package label. All study treatment products must be stored in a secure, limited-access location and may be dispensed only by the Investigator or by a member of the staff specifically authorized by the Investigator.

9.1.4. Study Treatment Administration

AG-348 and matching placebo tablets are to be taken orally and swallowed whole with water. The tablets are not to be crushed, chewed, or dissolved in water. Doses of study treatment may be taken with or without food. Subjects will take 3 tablets, each of a different size, twice a day, approximately 12 hours apart (ie, 12 hours ±2 hours), regardless of randomization to active or placebo and dose level (ie, 6 tablets total each day). Subjects will be instructed to complete a dosing diary each day in the eDiary dispensed during screening.

If a dose of study treatment is not taken 2 hours before or 2 hours after the scheduled dosing time, the dose should be skipped. If a dose of study treatment is skipped, the next dose should then be taken approximately 24 hours from the previous dose.

Subjects should be advised not to discontinue or interrupt dosing without first speaking with the treating Investigator except in case of medical emergency; abrupt discontinuation or interruption of AG-348 may result in withdrawal hemolysis. If a subject needs to

discontinue or interrupt study treatment at any time during the study, guidance is provided in Section 9.5.

9.1.5. Study Treatment Accountability

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

The Investigator or delegate will maintain accurate drug accountability records indicating the drug's delivery date to the site, inventory at the site, use by each subject, and return to the Sponsor, or the Sponsor's 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 treatment received from the Sponsor. Accountability records will include dates, quantities, batch/serial numbers, expiration dates (if applicable), and subject numbers. The Site Monitor will review drug accountability at the site on a schedule agreed to by the Sponsor.

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

All unused and used study treatment will be retained at the site until it is inventoried by the Site Monitor. All used, unused, or expired study treatment will be returned to the Sponsor, or the Sponsor's designee or, if authorized, disposed of at the study site per the site's Standard Operating Procedures and documented.

Study treatment is expected to be dispensed to the subject at the study site. Under exceptional circumstances and with agreement of the Sponsor (or representative), study treatment can be provided at the subject's home, if acceptable by practice and allowed by local regulations.

9.1.6. Study Treatment Handling and Disposal

All unused study treatment 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.

9.2. Randomization

Eligible subjects will be randomized 1:1 to receive either AG-348 or matching placebo. The randomization will be stratified by the average of screening Hb concentrations (<8.5 vs ≥8.5 g/dL [5.28 mmol/L]) and the *PKLR* gene mutation category (missense/missense vs missense/non-missense). The randomization assignment will be double-blinded to minimize any potential assessment bias. In rare instances where *PKLR* gene mutation category cannot be made definitively (eg, if a subject harbors 3 mutant *PKLR* alleles), the subject will be assigned to the missense/non-missense category.

An interactive response system (IXRS) will be used to assign subjects to treatment. The randomization code will be prepared and produced by a qualified randomization vendor. The Sponsor's study biostatistician will review and approve the final dummy randomization. The final (production) unblinded randomization will be reviewed by the statistician from the qualified randomization vendor and transferred to the IXRS vendor directly. The Sponsor's study team

will not have access to the final unblinded randomization until database lock and study unblinding.

9.3. Blinding

Blinding of treatment allocation to subjects, Investigators, and site personnel will be ensured by identical tablet appearance, and by identical labelling between placebo and active drug.

The subject, Investigators, and site personnel will be blinded to the subject's study treatment allocation until database lock, if that subject is not entering an extension study.

If the subject expresses his/her intention to enter an extension study, the subject, Investigators, and site personnel will be unblinded to the subject's study treatment allocation, but only after the subject completes the Week 24 assessments.

The Sponsor study team will be blinded to study treatment allocation until the database has been locked. The subject, Investigators and site personnel, and Sponsor study team will not have access to the pharmacokinetic data until the database has been locked.

9.3.1. Handling of Restricted Data

The following data will be considered restricted data: RBC parameters, hemolysis parameters, and hormone data. The Investigators will have access to these restricted data, with the exception of the hormone data, for their own subjects. Although Hb is evaluated as the primary efficacy endpoint, it is also considered part of the safety evaluation. For this reason, it is not justifiable to restrict the Investigators to the Hb results of the subjects whose welfare they are responsible for.

Subjects should not have access to their own restricted data, to reduce the potential for bias in the PRO measurements.

The Sponsor study team will not have access to the restricted data. The Independent Medical Monitor, or designee, will have access to the restricted data to be able to provide guidance to the Investigators and perform periodic review of these data. Additional details are provided in the data blinding/unblinding plan.

9.4. Unblinding

In the event of a medical emergency or pregnancy in a female subject, or in the female sexual partner of a male subject, in which knowledge of the treatment allocation is critical to the subject's management, the blind for that subject may be broken by the Investigator. *Prior to unblinding,* Investigators are encouraged to discuss a plan to break the blinding code with the Independent Medical Monitor, or designee.

The Investigator will be able to access the interactive response technology to reveal the identity of the treatment for that subject. The Investigator must record the nature of the emergency that required the unblinding, along with the date and time of the unblinding on the proper source documentation, and notify the Independent Medical Monitor, or designee, of the unblinding.

In the event that a subject's treatment assignment is unblinded to the subject, Investigator, or the Sponsor study team (not including the Safety Officer who is not part of the Sponsor study team and not involved in the current study conduct), either accidentally or in the case of emergency

unblinding, the subject will be allowed to continue study treatment, but the data after unblinding will not be included in the efficacy analyses.

9.5. Criteria for Dose Modification and Study Treatment Discontinuation or Interruption for Adverse Events or Excessive Hb Response

It is important that, as much as possible, a subject does not abruptly discontinue or interrupt study treatment due to the risk of withdrawal hemolysis. The Investigator will closely monitor all subjects for safety.

A subject's study treatment dose may be reduced (to 5 mg BID or 20 mg BID), interrupted, and/or discontinued; the possible scenarios in which study treatment reduction, interruption, and/or discontinuation may be considered include the following:

1. Excessive Hb response, with the subject's Hb concentration higher than 2 g/dL (1.24 mmol/L) below the ULN as applies to men and women
2. Occurrence of study treatment related AEs (except for excessive Hb response)
3. Planned study treatment discontinuation with the recommended dose taper (ie, subject withdraws consent for study treatment)

9.5.1. Excessive Hb Response

For a subject with an excessive Hb response as defined as higher than 2 g/dL (1.24 mmol/L) below the ULN up to and including the ULN, a dose decrease to the next lower dose level should be considered for the subject, without a need for a dose taper (ie, if the subject experiences an excessive Hb response at 50 mg BID, his/her dose will be decreased to 20 mg BID). The same rule applies to the decrease from 20 mg BID to 5 mg BID.

For a subject with an excessive Hb response higher than the ULN, the event should be reported as an AE and the subject's dose will be decreased to the next lower dose level, without a need for a dose taper (ie, if the subject experiences an excessive Hb response at 50 mg BID, his/her dose will be decreased to 20 mg BID). The same rule applies to the decrease from 20 mg BID to 5 mg BID.

9.5.2. Occurrence of Study Treatment Related Adverse Events (Except for Excessive Hb Response)

Dose modification which may be required due to study treatment related AEs (except for excessive Hb response) are described in Table 1.

Table 1: Dose Modification for Adverse Events Considered Related to Study Treatment (Except for Excessive Hb Response), Study AG348-C-006

Related Adverse Event(s) Severity	Dose Modification
Grade 1	None required.
Grade 2	None required. Contact the Independent Medical Monitor, or designee, to discuss specific cases that may need to be managed as Grade 3 events (see below).
Grade 3	After careful consideration of the relative risk of maintaining the subject on the study treatment versus the risk of withdrawal hemolysis when stopping treatment abruptly or reducing the dose, the Investigator should determine which of the following options is appropriate: • Maintaining the current dose, **or** • Performing the recommended dose taper, **or** • Stopping the study treatment abruptly If the decision is made to maintain the current dose of study treatment, then no dose changes are required. At least once weekly monitoring should be performed until the event resolves to baseline or Grade 1 (whichever is lower). If the event persists, performing the recommended dose taper or stopping the study treatment abruptly should be considered. If the decision is made to perform the recommended dose taper or to stop the study treatment abruptly, the below instructions should be followed for re-introduction or re-escalation of the study treatment, respectively. **In all cases, re-introduction and re-escalation of study treatment should be performed only after discussion with the Independent Medical Monitor, or designee.** • Restarting study treatment after dosing was stopped: – Once the event resolves to baseline or Grade 1 (whichever is lower) and the decision is made to restart treatment, the study treatment should be re-introduced at the 5 mg BID dose level. If the event does not re-occur after at least 4 weeks on 5 mg BID (with at least once weekly monitoring), the dose may be increased from 5 mg BID to 20 mg BID. If the event does not re-occur after at least 4 weeks on 20 mg BID (with at least once weekly monitoring), the dose may be increased from 20 mg BID to 50 mg BID. In both cases, dose escalation should follow the guidance in Section 7.1.3. • Events resolving during the recommended dose taper (ie, the subject is still on study treatment): – If during the recommended dose taper, the event resolves to baseline or Grade 1 (whichever is lower), the study treatment should be maintained at the dose at which the event resolved

Related Adverse Event(s) Severity	Dose Modification
	for at least 4 weeks (with at least once weekly monitoring). If the event does not re-occur after at least 4 weeks, then the dose may be increased to the next highest BID dose (5 mg BID, 20 mg BID, or 50 mg BID) with at least once weekly monitoring. If the event does not re-occur after at least 4 weeks (with at least once weekly monitoring), and the subject is not already receiving 50 mg BID, then an increase to the next BID dose level should be considered. In both cases, dose escalation should follow the guidance in Section 7.1.3. • Re-occurrence of the AE: – If the AE re-occurs at any point during the above scenarios, the subject should undergo the recommended dose taper or stop study treatment abruptly, if necessitated by the risk of the AE. If the subject undergoes the recommended dose taper and the AE resolves during the taper, study treatment should be maintained at the next lowest BID dose below the dose at which the AE resolved. If the subject cannot tolerate BID dosing, another regimen may be allowed after discussion with, and approval by, the Independent Medical Monitor, or designee. If the AE does not resolve to baseline or Grade 1 (whichever is lower) after the dose is decreased, a further decrease in the dose should be considered. If the AE still does not resolve, study treatment should be permanently discontinued.
Grade 4	After careful consideration of the relative risk of withdrawal hemolysis when stopping study treatment abruptly versus reducing the dose, the Investigator should determine which of the following options is appropriate: • Performing the recommended dose taper **or** • Stopping the study treatment abruptly If the event resolves, and the Investigator believes that re-introducing study treatment is justified, the Independent Medical Monitor, or designee, should be consulted before any further study treatment is administered.

Abbreviations: AE = adverse event; BID = twice daily; Hb = hemoglobin.

9.5.3. Study Treatment Discontinuation or Interruption With Recommended Dose Taper Regimen

All subjects who discontinue or interrupt study treatment during the study should undergo the recommended dose taper regimen in Table 2. This regimen is based on the study treatment dose administered to the subject at the start of the taper and occurs in 1 or 2 sequential steps. Subjects who were unblinded and allocated to placebo do not need to undergo the recommended dose taper when discontinuing or interrupting study treatment.

Subjects undergoing the recommended dose taper should be monitored as clinically indicated for signs of withdrawal hemolysis and worsening of anemia. If the recommended dose taper is performed in order to permanently discontinue study treatment, subjects stop taking the study treatment after the taper has been completed.

Table 2: Recommended Dose Taper Regimen, Study AG348-C-006

Starting Dose (at the time of the dose taper)	First Step ×7 days	Second Step ×7 days
5 mg BID	5 mg QD	---
20 mg BID	20 mg QD	5 mg QD
50 mg BID	50 mg QD	20 mg QD

Abbreviations: BID = twice daily; QD = once daily.

9.6. Treatment Compliance

Treatment compliance will be assessed by drug accountability (ie, number of tablets dispensed vs number returned).

9.7. Prior and Concomitant Medications

Prior medications are defined as those administered anytime within the 28 days prior to signing of the ICF until the first dose of study treatment and concomitant medications are defined as those administered from the point of first dose of study treatment through the subject's completion of the study. All prior and concomitant medications must be recorded in the appropriate section of the source documentation and eCRF along with any dosage information, dates of administration, mode of administration, and reason for use. For non-drug therapies, please reference Section 9.8.

9.7.1. Prohibited Medications

Concomitant use of investigational drugs is not allowed while subjects are participating in this study. All subjects must discontinue any investigational drug no less than 3 months prior to the first dose of study treatment.

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

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

Based on these results, below is a list of concomitant therapy to be avoided and concomitant therapy requiring careful monitoring.

The following are prohibited at all times during participation in this study:

- Strong inhibitors of CYP3A4 (listed in the AG-348 IB)

- Products known to inhibit CYP3A4, such as grapefruit or grapefruit juice
- Strong inducers of CYP3A4 (listed in the AG-348 IB)
- Strong inhibitors of P-gp (listed in the AG-348 IB)
- Digoxin, a P-gp transporter-sensitive substrate

If a subject is taking any medication listed in the AG-348 IB and/or digoxin prior to enrolling in the study, the medication must be discontinued at least 5 days or a time frame equivalent to 5 half-lives (whichever is longer) prior to Day 1 dosing.

- Hematopoietic stimulating agents (eg, erythropoietins, granulocyte colony stimulating factors, thrombopoietins) must be discontinued no less than 28 days prior to the first dose of study treatment. B12 injections are permitted for subjects with a prior diagnosis of B12 deficiency syndromes. Subjects 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 treatment.

The medications that fall under the categories mentioned below should be avoided and replaced with alternative treatments. If this is not possible, subjects receiving these medications should be carefully monitored. A general monitoring guideline for Investigators whose patients take medications that fall under the categories mentioned below is as follows: Investigators must monitor subjects for lack of efficacy of the prescribed medication or for side effects arising from the medication. If either a lack of efficacy of the prescribed medication or side effects suspected to be related to the prescribed medication are noticed, then the Investigator should make appropriate modifications to the dose of the prescribed medication or find alternatives to the prescribed medication.

- Corticosteroids (sensitive substrates of CYP3A4 and weak CYP3A4 inducers)
- Sensitive substrates of CYP3A4 (listed in the AG-348 IB)
- Moderate inhibitors of CYP3A4 (listed in the AG-348 IB)
- Sensitive substrates of CYP2B6 (listed in the AG-348 IB)
- Proton-pump inhibitors and H2-receptor antagonists (listed in the AG-348 IB). 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 28 days after their last dose of study treatment, as specified in Inclusion Criterion 9 (Section 8.1).

9.7.2. Allowed Concomitant Medications

Medications other than those specified above (Section 9.7.1) are permitted during the study. All intercurrent medical conditions will be treated at the discretion of the Investigator according to acceptable local standards of medical care. Subjects may receive analgesics, antiemetics,

anti-infectives, and antipyretics as medically indicated and consistent with the guidance in Section 9.7.1.

Subjects may continue iron chelation therapy with deferoxamine, deferasirox, or deferiprone. As iron overload is a long-term complication of PK deficiency, any initiation, completion, or change of iron chelation therapy will be of particular interest. Data about chelation therapy use will be carefully collected.

Subjects must continue taking at least 0.8 mg oral folic acid daily for the duration of the study.

9.8. Prior and Concomitant Non-Drug Therapies

Prior non-drug therapies determined to be relevant for medical/surgical history and/or eligibility criteria, such as major surgeries within 6 months prior to signing the ICF (see Section 8.2, Exclusion Criterion 9) and splenectomy within 12 months prior to signing the ICF (see Section 8.2, Exclusion Criterion 3) should be recorded.

Relevant concomitant non-drug therapies used to treat an AE should be collected from the signing of ICF until the subject completes the study.

9.8.1. Prior and Concomitant Transfusions

Transfusions administered within 12 months prior to the first day of study treatment should be recorded to verify that the subject meets the inclusion criterion for “not regularly transfused” (see Section 8.1, Inclusion Criterion 5)

For each transfusion administered from Day 1 until the end of the study, the date of transfusion and number of RBC units transfused will be recorded.

10. STUDY ASSESSMENTS

The timing of all study assessments is indicated in the Schedule of Assessments (Section 10.13).

10.1. Informed Consent

A description of the study is to be presented to each potential subject and a signed and dated ICF is to be obtained before any study-specific procedures are performed. The ICF will contain a separate section regarding the option to use leftover biological samples for analysis of additional biomarkers; subjects may opt-in or decline; this will not affect their eligibility for the study.

10.2. Study Eligibility

A subject's eligibility will be assessed and confirmed during the Screening Period. The Investigator will determine whether each subject meets all the inclusion criteria and none of the exclusion criteria. Eligibility of each subject will be confirmed by the Medical Monitor, or designee.

If a subject is deemed ineligible for the study due to a transient condition (eg, prohibited concomitant medication, curable medical condition), the subject may be rescreened after the criterion that made the subject ineligible has resolved. Subjects who were ineligible according to a previous version of the protocol may be rescreened if the subject could be eligible according to the current version of the protocol.

The following assessments will not need to be repeated at rescreening if they were performed correctly at the initial screening: demographics, medical/surgical history (unless new information needs to be added), liver MRI (unless done more than 3 months prior), DXA scan (unless done more than 3 months prior), and *PKLR* genotyping.

10.3. Demographics

Subject demographic data will include gender, year of birth, race, and ethnicity.

Race and ethnicity will be collected to ensure that any race and ethnicity related specificities in the safety, pharmacokinetics, and/or efficacy of AG-348 can be captured and interpreted accurately.

Collection of demographic data will be modified by country regulatory requirements, as appropriate.

10.4. Medical/Surgical History

All medical and surgical history deemed to be relevant per the Investigator (in particular, pertaining but not limited to the diagnosis of PK deficiency) and current medical conditions are to be recorded on the source documentation and included in the eCRF during screening.

Prior history of splenectomy and/or cholecystectomy must be documented and included in the eCRF during screening for all subjects.

10.4.1. Prior Transfusions

The dates of transfusions for the 12-month period prior to the first day of study treatment will be required for subject eligibility confirmation (Section 8.1, Inclusion Criterion 5).

10.4.2. Iron Overload-Related History

Historical use of chelation therapy for the 12 months prior to signing the ICF should be recorded, including type of iron chelation therapy, start date, stop date, and dose. Additionally, serum iron, ferritin, transferrin saturation, and LIC data should be recorded for the 12 months prior to signing the ICF.

10.5. Pyruvate Kinase Deficiency Specific Assessments

10.5.1. *PKLR* Genotyping

The *PKLR* genotyping and genotype classification (ie, missense or non-missense) will be performed during screening by a central genotyping laboratory for confirmation of study eligibility.

10.5.2. PK Enzyme Assay

The PK enzyme assay will be performed on the blood sample collected during screening by a central laboratory.

10.6. Assessment of Efficacy

10.6.1. Hemoglobin Laboratory Assessment

Hemoglobin concentrations will be collected to support efficacy (and safety) assessments. Blood will be drawn for hematology assessments as outlined in the Schedule of Assessments (Section 10.13).

10.6.2. Additional Laboratory Assessments for Efficacy

All the RBC parameters, haptoglobin, and LDH values will be collected to support additional efficacy assessments. Blood will be drawn for these assessments as outlined in the Schedule of Assessments (Section 10.13).

10.7. Assessments of Safety

10.7.1. Vital Signs

Vital signs will be recorded and will include systolic and diastolic BP, heart rate, and body temperature.

10.7.2. Physical Examination

Complete PEs will be performed according to the Schedule of Assessments (Section 10.13) and additionally when clinically indicated, at the discretion of the Investigator.

Height will be recorded only during screening.oh

10.7.3. Prior and Concomitant Medications

Prior and concomitant medications will be captured as described in Section 9.7 and per the Schedule of Assessments (Section 10.13).

10.7.4. Safety Laboratory Assessments

Safety laboratory assessments are described in Section 10.8.1.

10.7.5. Electrocardiogram

The 12-lead ECGs should be performed following 5 minutes of recumbence and in triplicate, using the ECG machine provided by the central vendor and according to the vendor manual. The ECG at Week 12 will be performed prior to, but within the same window as, the pharmacokinetic sample collected 1 hour (±5 minutes) after the study treatment dose administration to align with the approximate T_{max} (time to maximum [peak] concentration) of the study treatment.

The ECGs will be read promptly by a qualified physician at the study site to detect any eligibility or safety issue. Only QTcF (not heart rate-corrected QT interval by Bazett's method [QTcB]) will be used for determination of eligibility. In addition to the local read, the ECGs will be sent promptly to the central vendor for a data-analysis read.

An ECG will be repeated if clinically significant abnormalities are observed, if artifacts are present, or if machine/equipment errors occur.

10.7.6. Dual-Energy X-ray Absorptiometry Scans

The DXA scans of the lumbar spine and proximal femur (trochanter and inter-trochanter, which comprise the total hip, and femoral neck) will be performed according to the instructions provided by the central vendor (see vendor's manual for details). The DXA scans will be transmitted promptly to the central vendor for assessment of technical adequacy and may have to be repeated (before first dose for the screening DXA scan) if not technically adequate. The DXA scans will be read and interpreted by the central vendor.

10.7.7. Menstrual Cycle Diary

Menstruating female subjects will be required to fill out an electronic menstrual cycle diary for each menstrual period in order to detect any change in menstrual cycles. Subjects will record the start date, stop date, and any notable characteristics of each menstrual cycle in the eDiary provided at screening.

10.8. Laboratory Assessments

Laboratory assessment samples will be collected according to the Schedule of Assessments (Section 10.13). All blood samples are to be analyzed by the central laboratory. If results from the central laboratory are not available to support necessary clinical decision-making then results from a local laboratory may be used. Blood samples for the second Hb assessment during screening and at the Week 10 assessments may be collected outside of the study site and sent to the central laboratory by qualified personnel (eg, home health care nurse), if allowed by local regulations.

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, they are free to exercise their discretion to do so.

All clinically significant laboratory abnormalities noted on testing will be followed by repeat testing and further investigated according to the judgment of the Investigator.

Safety laboratory assessments and other laboratory assessments are listed in Section 10.8.1 and Section 10.8.2, respectively.

10.8.1. Safety Laboratory Assessments

The following safety laboratory parameters will be measured:

Hematology:	Complete blood count (hematocrit [HCT], Hb, RBC count, percent reticulocyte, absolute reticulocyte count [if available], immature reticulocyte fraction [IRF-H and IRF-M+H] [if available], MCV, mean corpuscular Hb, mean corpuscular Hb concentration, red cell distribution width, nucleated RBC count, white blood cell count, ANC, absolute lymphocyte count, eosinophil count, basophil count, absolute monocyte count, and platelet count).
Serum Chemistry:	Sodium, potassium, chloride, calcium, magnesium, phosphorus, carbon dioxide or bicarbonate, albumin, total protein, glucose, blood urea nitrogen or urea, uric acid, and creatinine. At screening, estimated GFR, measured GFR, or calculated (Cockcroft-Gault) CrCL will be assessed.
LFTs:	Alkaline phosphatase, ALT, AST, total bilirubin, direct bilirubin, and indirect bilirubin
Sex Steroid Testing:	Testosterone (total and free), estrone, and estradiol.
Fasting Lipids:	Total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides measured by standard method and nuclear magnetic resonance
Coagulation Studies:	Fibrinogen, aPTT, and INR
Dipstick Urinalysis:	Protein, glucose, leukocytes, and blood
Pregnancy Test (if applicable):	Serum (hCG) or urine pregnancy tests (with the exception of the screening pregnancy test which must be serum)
***UGT1A1* Genotyping:**	To detect subjects with Gilbert syndrome

10.8.2. Other Laboratory Assessments

A blood sample for serology, including HBsAg, HCVAb screen, and HIV-1 and HIV-2 Ab, will be collected from all subjects for eligibility criteria.

The following other laboratory parameters will be assessed:

- FSH (to confirm post-menopausal status)
- Iron panel and related markers (iron, serum ferritin, total iron-binding capacity [TIBC], transferrin saturation, non-transferrin bound iron [NTBI], hepcidin, C-reactive protein [CRP], and other markers of iron metabolism). Remaining sample may be used for analyses of lipoproteins (only in subjects who have agreed to this optional analysis in the ICF).
- Markers of erythropoietic activity: soluble transferrin receptor, erythropoietin (EPO), erythroferrone, and other markers of erythropoiesis
- Assessments of complications of iron overload: thyroxine, thyroid-stimulating hormone, parathormone, fructosamine, and Vitamin D

10.9. Other Assessments

Liver iron concentration will be measured by MRI based on the measurement and imaging of proton transverse relaxation rates (R2). The MRI data collected at the site will be transferred to a central vendor for analysis. For further details, please refer to the vendor's manual.

10.10. Pharmacokinetic Assessments

10.10.1. Blood Sample Collection

On days of pharmacokinetic blood sample collection, the morning dose of study treatment must be administered at the study site. On days where a predose sample is required, the study treatment must be administered after the predose sample is taken. Plasma samples for pharmacokinetic analysis of AG-348 will be collected at the following time points (note: time points are listed in relation to the morning dose of study treatment):

- Day 1 [predose (within 60 minutes prior to study treatment administration)]
- Week 2 (1-2 hours and 3-4 hours post study treatment administration)
- Week 6 (4-5 hours and 6-7 hours post study treatment administration)
- Week 12 (full profile blood sampling)
  - predose (within 60 minutes prior to study treatment administration)
  - 30 minutes (±5 minutes) post study treatment administration
  - 1 hour (±5 minutes) post study treatment administration
  - 2 hours (±5 minutes) post study treatment administration
  - 4 hours (±30 minutes) post study treatment administration
  - 8 hours (±30 minutes) post study treatment administration

- Week 16 [predose (within 60 minutes prior to study treatment administration)]

The actual date and time of sample collection will be recorded in the source documents and eCRF. An explanation should be provided in the source documents for any missed or mishandled pharmacokinetic samples, as well as for any samples collected outside the time windows.

10.10.2. Sample Analysis

Pharmacokinetic samples will be analyzed for AG-348 using a validated liquid chromatography-tandem mass spectrometry method. Remaining samples may be used for analyses of AG-348 metabolism (only in subjects who have agreed to this optional analysis in the ICF).

Plasma pharmacokinetic parameters will be computed, when data allow, using standard noncompartmental methods, based on observed plasma AG-348 concentrations and on actual sample collection times. These parameters will include, but may not be limited to, the following:

- $AUC_{0\text{-last}}$: The area under the plasma concentration × time curve from time 0 to the time of the last measurable concentration
- T_{last}: Time of last measureable concentration
- C_{max}: Maximum (peak) concentration
- T_{max}: Time to maximum (peak) concentration
- λ_z: Apparent terminal elimination rate constant, calculated from a semi-log plot of the plasma concentration versus time curve
- $t_{½}$: Terminal half-life
- CL/F: The apparent total plasma clearance (CLp) following oral (extravascular) dosing
- Vz/F: The apparent volume of distribution during the terminal elimination phase following oral (extravascular) dosing

10.11. Pharmacodynamic Assessments

Pharmacodynamic samples to measure PKR protein levels in whole blood will be taken at the Day 1 Visit before administration of the first dose and predose at Week 16. Additional analysis of exploratory biomarkers (PKM protein levels and levels of intermediates in the metabolic pathways affected by PKR) to further the understanding of the mechanism of action of AG-348 may be performed on leftover samples in subjects who have agreed to this optional analysis in the ICF.

Subjects in the study, contingent on clinical site feasibility, will have blood taken at the Day 1 Visit before administration of the first dose and predose at Week 12 for the PKR flux assay. The PKR flux assay measures the change in carbon flow from glucose through the PKR reaction to pyruvate and then to lactate after AG-348 treatment.

On days of sample collection, the morning dose of study treatment must be administered at the study site following the predose collections. Predose samples for pharmacodynamic assessments should be collected within 60 minutes prior to study treatment.

10.12. Health-Related Quality of Life Assessments and CGIC

Patient-reported outcome measures, specifically PKDD and PKDIA, will be evaluated during screening and throughout the study. Several other HRQoL measures have been included as validators (including relevant subscales) in this study to allow for in-trial validation of the PKDD and PKDIA. The exact methods and techniques to be used for PKDD and PKDIA calibration and scoring will be included in the formal PRO calibration and validation statistical analysis plan (SAP). The following measures will be used for validation of the PKDD and PKDIA:

- EQ-5D-5L
- The 12-item Short Form Health Survey, Version 2 (SF-12v2)
- FACT-An – "Additional Concerns"
- Patient Global Impression of Severity (PGIS)
- Patient Global Impression of Change (PGIC)
- Clinician Global Impression of Change (CGIC)

Subjects will use the eDiary (provided during screening) to record responses to each of the HRQoL assessments (Section 10.12.1 through Section 10.12.7). The HRQoL assessments should be completed in the evening (between 5:00-11:00 PM) within the window for the relevant study visit as indicated in the Schedule of Assessments (Section 10.13).

10.12.1. Pyruvate Kinase Deficiency-Specific HRQoL Assessments

The PKDD is a 7-item PRO measure of the core signs and symptoms associated with PK deficiency in adults. Subjects rate their experience with symptoms of PK deficiency on the present day. The symptoms include those associated with tiredness, jaundice, bone pain, shortness of breath, and energy level.

The PKDIA is a 12-item PRO measure of the common impacts of PK deficiency on activities of daily living. Subjects rate how PK deficiency has impacted aspects of daily living in the past 7 days, including impacts on relationships; perceived appearance; work performance; and leisure, social, mental, and physical activities.

These 2 tools were developed by the Sponsor to systematically assess and capture changes in symptom burden and impact on HRQoL.

10.12.2. European Quality of Life Five-Dimensional Descriptive System

The EQ-5D-5L is a standardized instrument for evaluating QoL over 5 dimensions: mobility, self-care, usual activities, pain, and mood. It is applicable over a broad range of health conditions and is used widely in clinical trials.

10.12.3. Patient Global Impression of Severity

The PGIS is a single-item questionnaire used to rate the subject's impression of the severity of his/her condition.

10.12.4. 12-Item Short Form Healthy Survey, Version 2

The SF-12v2 is a widely used generic measure of health status and measures 8 concepts of health: physical functioning, role limitations due to physical health problems, bodily pain, general health, vitality (energy/fatigue), social functioning, role limitations due to emotional problems, and mental health (psychological distress and psychological well-being).

10.12.5. Functional Assessment of Cancer Therapy Anemia – "Additional Concerns"

The FACT-An is a questionnaire developed within the Functional Assessment of Chronic Illness Therapy (FACIT) system. The FACT-An – "Additional Concerns" is a 20-item QoL measure for assessing fatigue and anemia-related concerns.

10.12.6. Patient Global Impression of Change

The PGIC is a self-reported measure that reflects a subject's perspective on his or her overall health status with respect to the study treatment. The assessment rates the subject's perceived overall improvement while receiving study treatment.

10.12.7. Clinician Global Impression of Change

The CGIC is an assessment completed by the study Investigator to provide the clinician's view of the subject's overall improvement and health status prior to and after initiating study treatment.

10.13. Schedule of Assessments

The Schedule of Assessments is in Table 3.

Table 3: Schedule of Assessments, Study AG348-C-006

	Screening Period	Dose Optimization Period (Part 1)							Fixed Dose Period (Part 2)			Follow-up Visit[5]
Visit:	**SCRN[1]**	**D1[2]**	**W2**	**W4**	**W6**	**W8**	**W10[3]**	**W12**	**W16**	**W20**	**W24/End of Study[4]**	**28D after last dose[5]**
Study Day:	**D -42 to D -1**	**1**	**15**	**29**	**43**	**57**	**71**	**85**	**113**	**141**	**169[4]**	
Visit Window:		**0**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±4 D**	**±4 D**
Procedures:												
Informed consent	X											
Pyruvate kinase enzyme assay	X											
PKLR genotyping	X[33]											
UGT1A1 genotyping[6]		X										
Demographics	X											
Medical/surgical history[7]	X	X										
Prior Medications[8]	X	X										
Eligibility confirmation[9]	X											
Randomization[9]		X										
Liver MRI to assess LIC[10]	X										X	
PE[11]	X	X						X			X	
Vital signs[12]	X	X	X	X	X	X		X	X	X	X	X
12-lead ECG[13]	X	X						X			X	X
DXA Scan[10]	X										X	
Dispense eDiary[14]	X											
Menstrual Cycle Diary[14]	X											
HRQoL Assessments[14]												
EQ-5D-5L	X[34]							X			X	

	Screening Period	Dose Optimization Period (Part 1)							Fixed Dose Period (Part 2)			Follow-up Visit[5]
Visit:	**SCRN[1]**	**D1[2]**	**W2**	**W4**	**W6**	**W8**	**W10[3]**	**W12**	**W16**	**W20**	**W24/End of Study[4]**	**28D after last dose[5]**
Study Day:	**D -42 to D -1**	**1**	**15**	**29**	**43**	**57**	**71**	**85**	**113**	**141**	**169[4]**	
Visit Window:		**0**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±4 D**	**±4 D**
Procedures:												
SF-12v2	X[34]							X			X	
PKDD[14]	X–Daily–X	X–Daily–X										
PKDIA	X[34]			X		X		X	X	X	X	
FACT-An – "Additional Concerns"	X[34]							X			X	
PGIS	X[34]			X		X		X			X	
PGIC				X		X		X			X	
CGIC				X		X		X			X	
Dosing eDiary[14]		X										
Return eDiary[15]											X[4]	X
Clinical Laboratory Evaluations[16]												
HBsAg, HCVAb, HIV-1 and -2 Ab[17]	X											
FSH[18]	X											
Hematology[19]	X, X[35]	X	X	X	X	X	X	X	X	X	X	X
Haptoglobin and LDH	X	X	X	X	X	X	X	X	X	X	X	X
Serum chemistry[20]	X	X						X			X	X
LFTs[21]	X	X	X	X	X	X	X	X	X	X	X	X
Coagulation studies[22]	X	X						X			X	
Urinalysis[23]	X	X						X			X	

	Screening Period	Dose Optimization Period (Part 1)							Fixed Dose Period (Part 2)			Follow-up Visit[5]
Visit:	**SCRN[1]**	**D1[2]**	**W2**	**W4**	**W6**	**W8**	**W10[3]**	**W12**	**W16**	**W20**	**W24/End of Study[4]**	**28D after last dose[5]**
Study Day:	**D -42 to D -1**	**1**	**15**	**29**	**43**	**57**	**71**	**85**	**113**	**141**	**169[4]**	
Visit Window:		**0**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±4 D**	**±4 D**
Procedures:												
Pregnancy test[24]	X	X		X		X		X	X	X	X	X
Iron panel and related markers[25]	X	X						X			X	
Markers of erythropoietic activity[25]	X	X						X			X	
T4, TSH, PTH, Fructosamine, and Vitamin D		X						X			X	
Lipids[26]	X	X		X		X		X	X	X	X	X
Sex Steroids[27]		X		X		X		X	X	X	X	X
PD Assessments[28]												
PKR protein		X							X			
PKR flux assay		X						X				
Dispense study treatment[29]		X		X		X		X	X	X	X[4]	
Study treatment dosing		X										
Return study treatment				X		X		X	X	X	X[4]	X
Pharmacokinetic Assessments												
Pharmacokinetic sparse blood sampling[30]		X	X		X				X			
Pharmacokinetic full-profile blood sampling[31]								X				
SAEs/AEs/AESIs[32]	X											

	Screening Period	Dose Optimization Period (Part 1)							Fixed Dose Period (Part 2)			Follow-up Visit[5]
Visit:	**SCRN[1]**	**D1[2]**	**W2**	**W4**	**W6**	**W8**	**W10[3]**	**W12**	**W16**	**W20**	**W24/End of Study[4]**	**28D after last dose[5]**
Study Day:	**D -42 to D -1**	**1**	**15**	**29**	**43**	**57**	**71**	**85**	**113**	**141**	**169[4]**	
Visit Window:		**0**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±4 D**	**±4 D**
Procedures:												
Concomitant medications/transfusions		X										

Abbreviations: Ab = antibody; AE = adverse event; AESI = adverse event of special interest; ALC = absolute leukocyte count; ALP = alkaline phosphatase; ALT = alanine aminotransferase; ANC = absolute neutrophil count; aPTT = activated partial thromboplastin time; AST = aspartate aminotransferase; BP = blood pressure; BUN = blood urea nitrogen; CBC = complete blood count; CGIC = Clinical Global Impression of Change; CO_2 = carbon dioxide; CrCL = creatinine clearance; CRP = C reactive protein; D = day; DXA = dual-energy x-ray absorptiometry; ECG = electrocardiogram; eDiary = electronic diary; EPO = erythropoietin; EQ-5D-5L = European quality of life five-dimensional descriptive system; FACT-An = Functional Assessment of Cancer Therapy-Anemia; FSH = follicle-stimulating hormone; GFR = glomerular filtration rate; Hb = hemoglobin; HBsAg = hepatitis B surface antigen; hCG = human chorionic gonadotropin; HCT = hematocrit; HCV = hepatitis C virus; HDL-C = high-density lipoprotein cholesterol; HIV = human immunodeficiency virus; HRQoL = health-related quality of life; ICF = informed consent form; INR = international normalized ratio; LDH = lactate dehydrogenase; LDL-C = low-density lipoprotein cholesterol; LFT = liver function test; LIC = liver iron concentration; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MRI = magnetic resonance imaging; NRBC = nucleated red blood cells; NTBI = non-transferrin bound iron; PD = pharmacodynamics; PE = physical examination; PGIC = Patient Global Impression of Change; PGIS = Patient Global Impression of Severity; PKDD = Pyruvate Kinase Deficiency Diary; PKDIA = Pyruvate Kinase Deficiency Impact Assessment; PKR = red blood cell-specific form of pyruvate kinase; PTH = parathyroid hormone; RBC = red blood cell; RDW = red cell distribution width; RT-PCR = reverse transcriptase-polymerase chain reaction; SAE = serious adverse event; SCRN = Screening; SF-12v2 = 12-item Short Form Health Survey, version 2; T4 = thyroxine; TIBC = total iron-binding capacity; TSH = thyroid-stimulating hormone; UGT1A1 = uridine diphosphate glucuronosyl transferase 1A1; ULN = upper limit of normal; W = week; WBC = white blood cell.

[1] A subject's Screening Period duration may be extended beyond 42 days upon the Medical Monitor's, or designee's, approval.

[2] The first dose of study treatment on Day 1 should be taken at the study site following all Day 1 assessments (with the exception of eDiary assessments; these assessments should be performed in the evening of Day 1).

[3] The Week 10 Visit will consist of laboratory assessments.

[4] Subjects who continue study treatment through Week 24 should continue taking study treatment at least through the morning dose of the Week 24 Visit. All subjects who remain on study during Part 2 through the Week 24 Visit may be eligible for an open-label extension study, in which all subjects will receive AG-348. Subjects who discontinue the study prior to the Week 24 Visit should attend the End of Study Visit 28±4 days after the last study visit that the subject attended or 28±4 days after the last dose of study treatment (including the recommended dose taper), whichever is later. Note, this may occur at any time during the study (Part 1 and Part 2). This visit will be identical to the Week 24 Visit with these exceptions: no study treatment will be dispensed at the End of Study Visit, and if the subject has not already done so, they should return their eDiary and remaining study treatment. These subjects do not need to complete the Follow-Up Visit.

[5] Subjects who continue study treatment through the Week 24 Visit but do not continue on into an extension study, should undergo the recommended dose taper and then attend the Follow-up Visit 28±4 days after the last dose of study treatment (including the recommended dose taper). Subjects who continue on into an extension study at the Week 24 Visit will not be required to attend the Follow-up Visit. Subjects who initiate or are undergoing the recommended dose taper, and for whom participation in an extension study remains undetermined, should perform the recommended dose taper as detailed in Section 9.5.3. Transition to an extension study should be discussed with the Independent Medical Monitor, or designee.

[6] *UGT1A1* genotyping results are not required prior to randomization.

[7] Transfusion history in the 12-month period prior to first day of study treatment should be reviewed for eligibility assessment. Subjects who received any transfusions within the 3 months prior to the first day of study treatment are not eligible. Additionally, serum iron, ferritin, transferrin saturation, and LIC data should be recorded for the 12 months prior to signing the ICF. Historical use of chelation therapy for the 12 months prior to signing the ICF should be recorded, including type of iron chelation therapy, start date, stop date, and dose.

[8] Prior medications are defined as those administered anytime within the 28 days prior to signing of the ICF until the first dose of study treatment.

[9] The Investigator will determine whether each subject meets all the inclusion criteria and none of the exclusion criteria. Eligibility of each subject will be confirmed by the Medical Monitor, or designee, during screening. Randomization can occur up to 24 hours prior to Day 1.

[10] The DXA and liver MRI should be performed per the vendor manuals. If the screening DXA and/or MRI scan(s) are deemed to be of low quality by the central vendors, the scan(s) will be repeated before the first dose of study treatment is administered.

[11] A complete PE will be performed. Height will be collected during screening only. Additional PEs may be performed when clinically indicated, at the discretion of the Investigator.

[12] Vital signs of systolic and diastolic BP, heart rate, and body temperature will be collected.

[13] The 12-lead ECGs will be conducted using the equipment provided by the vendor and according to the vendor manual, after 5 minutes of recumbence and in triplicate. The ECGs will be read promptly by a qualified physician at the study site to detect any eligibility or safety issue. In addition to the local read, the ECGs will be sent promptly to the central vendor for a data-analysis read. The ECG at Week 12 will be performed prior to but within the same window as the pharmacokinetic sample collected 1 hour (±5 minutes) after the study treatment dose administration to align with the approximate T_{max} of the study treatment.

[14] All subjects will be given an eDiary to record responses to HRQoL assessments and dosing. In addition, menstruating female subjects will record their menstrual cycles (start date, stop date, and notable characteristics) in the eDiary; the menstrual diary can be completed at any time of day. The PKDD assessment will be collected daily throughout screening, Part 1, and Part 2. The HRQoL assessments should be completed in the evening (between 5:00-11:00 PM) within the window for the relevant study visit. The dosing diary should be completed every day in the evening (between 5:00-11:00 PM).

[15] Subjects should return their eDiary once they complete the study. Subjects who discontinue early and attend the End of Study Visit should return their eDiary at this visit. Subjects should return their eDiary at their last visit of this study.

[16] On Day 1 of Part 1, blood samples for all clinical laboratory assessments should be collected predose. All clinical laboratory evaluations should be analyzed centrally. If results from the central laboratory are not available, to support necessary clinical decision-making, then results from a local laboratory may be used. For a safety issue that may require local laboratory evaluations, refer to Section 10.8.

[17] If the subject is positive for HCVAb, an RT-PCR test will be conducted. The subject is ineligible to enroll if active hepatitis C is present. The subject may be rescreened after receiving appropriate hepatitis C treatment.

[18] The FSH assessment will be performed only at screening for female subjects for confirmation of postmenopausal status (ie, female subjects who have not menstruated at all for at least the preceding 12 months prior to signing informed consent). Samples should be drawn in the morning (does not need to be fasting).

[19] Hematology parameters (ie, CBC with differential) include HCT, Hb, RBC count, percent reticulocyte, absolute reticulocyte count (if available), immature reticulocyte fraction (IRF-H and IRF-M+H) (if available), MCV, MCH, MCHC, RDW, NRBC, WBC count, ANC, ALC, eosinophil count, basophil count, absolute monocyte count, and platelet count.

[20] Serum chemistry parameters include sodium, potassium, chloride, calcium, magnesium, phosphorus, CO_2 or bicarbonate, albumin, total protein, glucose, BUN or urea, creatinine, and uric acid. At screening, estimated GFR, measured GFR, or calculated (Cockcroft-Gault) CrCL will be assessed.

[21] Liver function tests include ALP, ALT, AST, and total, direct, and indirect bilirubin.

[22] Coagulation studies include fibrinogen, aPTT, and INR.

[23] Urinalysis will be performed by a dipstick method and include assessments of protein, glucose, leukocytes, and blood.

[24] A serum (hCG) pregnancy test must be performed during screening. A urine or serum (hCG) pregnancy test must be performed and documented to be negative on Day 1, before administration of the first dose of study treatment. A urine or serum (hCG) pregnancy test must be repeated every 4 weeks at the indicated visits and must also be performed at any point throughout the study if pregnancy is clinically suspected. All pregnancy tests, other than the test performed at screening, should be performed locally.

[25] The iron panel and related markers include iron, serum ferritin, TIBC, transferrin saturation, NTBI, hepcidin, CRP, and other markers of iron metabolism. Erythropoietin, erythroferrone, soluble transferrin receptor, and other markers of erythropoiesis will be collected as markers of erythropoietic activity. Remaining sample may be used for analyses of lipoproteins (only in subjects who have agreed to this optional analysis in the ICF).

[26] For lipid testing samples for total cholesterol, LDL-C, HDL-C, and triglyceride will be collected after an overnight fast (see central laboratory vendor manual for detail). If the subject reports that they did not adhere to an overnight fast, these samples should not be collected at the scheduled visit. Instead, these samples should be collected within 2 weeks of the original time point at the next scheduled visit or at an unscheduled visit, after the subject has adhered to an overnight fast.
[27] Sexual steroid testing includes estrone, estradiol, and testosterone (total and free). Samples should be drawn in the morning (does not need to be fasting).
[28] Whole blood samples for PKR flux assay (contingent upon site feasibility) and samples for PKR protein are to be collected predose (within 60 minutes prior to study treatment administration). On days of sample collection, the morning dose of study treatment must be administered at the study site following the predose collections.
[29] Study treatment is expected to be dispensed to the subject at the study site. Under exceptional circumstances and with agreement of the Sponsor (or representative), study treatment can be provided at the subject's home, if acceptable by practice and allowed by local regulations. Subjects who discontinue or interrupt study treatment should undergo the recommended dose taper (Section 9.5.3), unless an emergency situation justifies discontinuing or interrupting the study treatment abruptly; study treatment will be dispensed to these subjects until study treatment is completely stopped. Following full discontinuation of study treatment, it will no longer be dispensed at any subsequent study visits the subject may attend.
[30] Pharmacokinetic sparse blood sampling collection times are as follows: Day 1 (predose; within 60 minutes prior to study treatment administration), Week 2 (1-2 hours and 3-4 hours post-study treatment administration), Week 6 (4-5 hours and 6-7 hours post-study treatment administration), and Week 16 (predose; within 60 minutes prior to study treatment administration). On days of pharmacokinetic blood sample collection, the morning dose of study treatment must be administered at the study site; on days where a predose sample is required, the study treatment must be administered after the predose sample is taken.
[31] Pharmacokinetic full profile blood sampling will be conducted in all subjects at the Week 12 Visit at the following time points: predose (within 60 minutes prior to study treatment administration) and 30 minutes (±5 minutes), 1 hour (±5 minutes), 2 hours (±5 minutes), 4 hours (±30 minutes), and 8 hours (±30 minutes) post study treatment administration. On days of pharmacokinetic blood sample collection, the morning dose of study treatment must be administered at the study site; on days where a predose sample is required, the study treatment must be administered after the predose sample is taken.
[32] The Investigator should ask the subject for information regarding sleep patterns, signs, and symptoms associated with insomnia.
[33] For eligibility confirmation, the PKR genotyping must be analyzed by the central laboratory; therefore, this must be drawn early in the Screening Period to ensure results are back in time to allow the Investigator to assess eligibility.
[34] PKDIA, FACT-An, SF12v2, EQ-5D-5L, and PGIS should all be completed on the first day of screening (+1 day) and then at weekly intervals (±1 day) until Part 1 Day 1.
[35] During screening, at least 2 hematology samples must be collected at least 7 days apart.

11. ADVERSE EVENTS

11.1. Reporting Period for Adverse Events and Serious Adverse Events

Monitoring of AEs, including frequency, severity, and characterization of 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 the time of the signing of ICF through the subject's completion of study or withdrawal of consent, whichever occurs first.

All AEs will be monitored until resolution of the AE to baseline, the AE is considered stable within the context of the trial, the subject is lost to follow-up, or until 28 days after the last dose of study treatment unless the subject is enrolled in an extension study, in which case ongoing AEs will be reported in the extension study database following consenting of the subject.

All SAEs will be followed until final outcome of the SAE is known, the subject is lost to follow-up, or the subject is enrolled in an extension study, in which case the SAE follow-up information will be reported in the extension study database. Any SAEs that are assessed as related to study treatment that occur ≥28 days post-treatment are to be reported to the Sponsor directly by the Investigator.

Adverse events will be evaluated by the Investigator and recorded as per Section 11.3. 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.

11.2. Definition of Adverse Events

11.2.1. Adverse Event

A clinical AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with the study treatment. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Pre-existing conditions that worsen during a study are to be reported as AEs. Withdrawal hemolysis is to be reported as a study treatment-related AE.

11.2.2. Serious Adverse Event

An AE or suspected adverse reaction is considered serious 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; but it does not include a reaction that 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 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.

11.2.3. Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (eg, cascade events or clinical sequelae) should be identified by their primary cause, with the exception of severe or serious secondary events. However, medically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the AE eCRF. Please refer to the eCRF completion guidance for examples of how to record events occurring secondary to other events.

All AEs should be recorded separately on the Adverse Event eCRF if it is unclear as to whether the events are associated.

11.2.4. Pre-existing Medical Conditions

A pre-existing medical condition is one that is present during screening for this study. Such conditions should be recorded on the Medical History eCRF.

A pre-existing medical condition should be recorded as an AE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on the Adverse Event eCRF, it is important to convey the concept that the pre-existing condition has changed by including applicable descriptors (eg, “more frequent headaches”).

11.2.5. Abnormal Laboratory Values

Abnormal laboratory tests should be repeated as soon as possible for confirmation. Not every laboratory abnormality qualifies as an AE. A laboratory test result should be reported as an AE if it meets any of the following criteria:

- Accompanied by clinical symptoms
- Results in a change in study treatment (eg, dosage modification, treatment interruption, or treatment discontinuation)
- Results in a medical intervention (eg, potassium supplementation for hypokalemia) or a change in concomitant therapy
- Clinically significant in the Investigator's judgment

It is the Investigator's responsibility to review all laboratory findings. Medical and scientific judgment should be exercised in deciding whether an isolated laboratory abnormality should be classified as an AE.

If a clinically significant laboratory abnormality is a sign of a disease or syndrome, only the diagnosis should be recorded on the Adverse Event eCRF.

If a clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded on the Adverse Event eCRF, along with a descriptor indicating if the test result is above or below the normal range.

11.2.6. Adverse Events of Special Interest

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

11.2.6.1. Transaminase Increase

Transaminase increase is an AESI for AG-348. In the event of a transaminase increase of >2.5 × baseline (defined as the mean of the screening and Day 1 values) 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 (defined as the mean of the screening and Day 1 values). 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 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 in the first time.

The Investigator should refer to Section 9.5 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 Independent Medical Monitor, or designee.

11.3. 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.

The Investigator should ask the subject for information regarding sleep patterns, signs, and symptoms associated with insomnia.

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, PE, or other diagnostic procedures will be recorded on the appropriate page of the eCRF. Any clinically relevant deterioration in laboratory assessments or other clinical finding is considered an AE and must be recorded on the appropriate pages of the eCRF. 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. All SAEs are to be reported within 24 hours from the point in time when the Investigator becomes aware of the SAE on the appropriate page of the eCRF. All SAEs must be reported whether or not they are considered causally related to the study treatment.

In the event that the electronic data capture (EDC) system is unavailable, a paper SAE and fax coversheet should be completed and faxed/emailed to the Sponsor within no more than 24 hours after learning of the event using the contact details provided to Investigators in the Serious Adverse Event Report Form Completion Guidelines.

Excessive Hb responses should only be reported as an AE if they meet the criteria for Hb increased per CTCAE (ie, Hb concentration is higher than the subject's ULN, as applies to men and women). Any reports of excessive Hb response should be graded using the CTCAE grading system.

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.

11.3.1. Intensity

The intensity of all AEs will be graded according to the NCI CTCAE. It is important to distinguish between SAEs and AEs with a severe intensity. An AE of severe intensity may not be considered serious. Severity is a measure of intensity, whereas seriousness is defined by the criteria in Section 11.2.2. For example, a severe headache without any further findings would not be considered an SAE. Alternatively, a mild presentation of a serious event, such as a myocardial

infarction assessed as mild by a cardiologist, that leads to hospitalization would be considered an SAE.

Severity of all AEs, including clinically significant treatment-emergent laboratory abnormalities, will be graded according to the NCI CTCAE v4.03. Adverse events not listed by the CTCAE will be graded as follows:

- Mild (Grade 1): The event is noticeable to the subject but does not interfere with routine activity.
- Moderate (Grade 2): The event interferes with routine activity but responds to symptomatic therapy or rest.
- Severe (Grade 3): The event significantly limits the subject's ability to perform routine activities despite symptomatic therapy.
- Life-threatening (Grade 4): An event in which the subject was at risk of death at the time of the event.
- Fatal (Grade 5): An event that results in the death of the subject.

11.3.2. Relationship to Study Treatment

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

- Not Related: AEs will be considered related, unless they fulfill the criteria as specified below:
  - Evidence exists that the AE has an etiology other than the study treatment (eg, pre-existing medical condition, underlying disease, intercurrent illness, concomitant medication); and/or
  - The AE has no plausible temporal relationship to the administration of the study treatment (eg, cancer diagnosed 2 days after the first dose of study treatment).
- Related: AEs will be considered related if they fulfill the criteria as specified below:
  - There is a plausible temporal relationship between the onset of the AE and administration of the study treatment, and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or
  - The AE follows a known pattern of response to the study treatment; and/or
  - The AE abates or resolves upon discontinuation of the study treatment or dose reduction and, if applicable, reappears upon re-challenge.

11.4. 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 28 days following the last dose of study treatment must be reported to the Independent Medical Monitor, or designee, within 24 hours of being notified of the pregnancy. Any pregnancy in a female sexual partner of

a participating male subject that occurs during this study or within 90 days following the last dose of study treatment, must be reported to the Independent Medical Monitor, or designee, within 24 hours of being notified of the pregnancy, if acceptable by practice and allowed by local regulations. If the pregnancy in a female sexual partner of a participating male subject occurs after the male subject has completed the study, the pregnancy must be reported to the Independent Medical Monitor, or designee, directly by the Investigator.

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

All outcomes of pregnancy in a female study participant or consented female sexual partner of a male participant must be reported by the Investigator to the Sponsor or Sponsor's designee on a Pregnancy Outcome Report form within 28 days after he/she has gained knowledge of the delivery or elective abortion.

Any SAE that occurs during pregnancy in a female study participant or consented female sexual partner of a male participant 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.

Women of reproductive potential as well as men with partners who are women of reproductive potential, must agree to be abstinent as part of their usual lifestyle, or agree to use 2 forms of contraception, 1 of which must be considered highly effective, from the time of giving informed consent, during the study, and for 28 days following the last dose of study treatment for women and 90 days following the last dose of study treatment for men. Periodic abstinence (eg, calendar, symptothermal, and post-ovulation methods) and withdrawal are not acceptable methods of contraception.

12. STATISTICAL METHODS

The study data will be analyzed and reported when all subjects have completed their Week 24 Visit or Follow-up Visit (if applicable) or have discontinued the study. Final analysis of all data, including efficacy and safety, will be performed by the Sponsor, or designee. A detailed analysis plan for the analysis of efficacy and safety data will be presented in a SAP, which will be finalized before the database lock and study unblinding.

12.1. Analysis Sets

Three analysis sets will be defined for evaluation of the study endpoints: Full Analysis Set (FAS), Per Protocol Set (PPS), and Safety Analysis Set (SAS).

12.1.1. Full Analysis Set

The FAS is defined as all subjects who are randomized. Subjects will be analyzed according to the treatment they were randomized to, regardless of any errors in dosing. The FAS will be the primary analysis set for the efficacy endpoints.

12.1.2. Per Protocol Set

The PPS is defined as all subjects who are randomized and dosed and have Hb assessments at Weeks 16, 20, and 24 during the Fixed Dose Period. Subjects will be analyzed according to the treatment to which they were randomized, regardless of any errors in dosing. The PPS will be used for a sensitivity analysis for the primary endpoint and the key secondary endpoint as detailed in Section 12.2.4.2.

12.1.3. Safety Analysis Set

The SAS includes all subjects who received at least 1 dose of study treatment. Subjects will be analyzed according to the actual treatment they received (eg, if a subject received any active treatment, the subject will be grouped to the active arm). The SAS will be the primary analysis set for the safety analyses.

12.2. Statistical Analysis

This section presents a summary of the planned analyses of efficacy and safety for this study. Additional supportive and exploratory analyses will be specified in the final SAP, which will be finalized before the database lock and study unblinding.

12.2.1. General Methods

All individual subject data for all subjects will be presented in data listings.

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

Categorical variables will be summarized using counts and percentages.

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 the study treatment.

- The individual subject's baseline Hb concentration is defined as the average of all available Hb concentrations from the central laboratory for that subject during the Screening Period up to the first dose of study treatment.
- For transaminase (AST and ALT) assessments, the baseline will be defined as the average of the Screening Period and Day 1 values.

Change (Absolute Change) from baseline: will be calculated as postbaseline value – baseline value.

Relative change from baseline: will be calculated and presented in percentage as 100 × (postbaseline value – baseline value)/baseline value.

Treatment-emergent period: is defined as the time from the first dose of study treatment to 28 days after the last dose of study treatment.

12.2.2. Background Characteristics

Subject disposition, demographic and baseline characteristics, prior and concomitant medications, study treatment exposure, and other background characteristics will be summarized. All subject data will be presented in subject data listings. All summaries will be based on the FAS unless otherwise specified in the SAP.

12.2.2.1. Subject Disposition

The number and percentage of subjects in different analysis sets below will be summarized, with the percentage calculated using the number of all subjects.

- Subjects randomized or dosed
- Randomized (FAS)
  - Randomized but not dosed
  - Randomized and dosed
- PPS
- Dosed (SAS)

The number and percentage of subjects in the following disposition categories will also be summarized. The percentage will be calculated based on the FAS.

- Completed treatment
- Prematurely discontinued treatment and the reasons for treatment discontinuations
- Completed study
- Prematurely discontinued the study and the reasons for study discontinuations

12.2.2.2. Demographics and Baseline Characteristics

Demographics, background (eg, medical history), and baseline characteristics will be summarized and presented for the FAS.

12.2.2.3. Prior and Concomitant Medications

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

- **Prior medication:** any medication that started before initial dosing of study treatment, regardless of when it ended
- **Concomitant medication:** medication continued or newly received during the treatment-emergent 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, during, or after the treatment-emergent period, it will be considered as prior, concomitant, and post-treatment medications.

Prior medications and concomitant medications will be summarized descriptively based on the FAS. Prior medications, concomitant medications and post-treatment medications will all be listed for each subject.

12.2.3. Study Treatment Exposure and Compliance

12.2.3.1. Study Treatment Exposure

Duration of study treatment exposure is defined as follows: last dose date – first dose date + 1 day, regardless of any interruptions in dosing. If the last dose date of study treatment is missing, the subject's treatment discontinuation or treatment completion date will be used for analysis purposes.

Duration of study treatment exposure will be summarized descriptively as a quantitative variable (number, mean, SD, median, minimum, and maximum).

Exposure summaries will be based on the FAS.

12.2.3.2. Study Treatment Compliance

Study treatment compliance will be assessed by percentage of days on treatment and percentage of tablets taken.

The percentage of days on treatment will be calculated as follows:

100 × (1 – [total number of days on study treatment] / [duration of full study treatment exposure + total number of days study treatment interrupted after last dose, if any]).

The total number of days of study treatment interruption is defined as the sum of (the number of days of each study treatment interruption), where number of days of each study treatment interruption is defined as the interruption end date − the corresponding interruption start date + 1.

Percent of tablets taken will be calculated as follows:

- 100 × (Total number of tablets administered) / (Expected number of tablets taken during the study)

Both percentage of days on treatment and percentage of tablets taken will be summarized descriptively as quantitative variables (number, mean, SD, median, minimum, and maximum). The number and percentage of subjects whose compliance is <80% or ≥80% will be summarized.

Study treatment compliance will be based on the FAS.

12.2.4. Efficacy Analysis

Unless specified otherwise, only central laboratory results will be considered in the Hb related efficacy analyses. Hemoglobin results collected within 2 months (61 days) after an RBC transfusion will be excluded.

12.2.4.1. Analysis of Primary Endpoint

In the primary analysis of the primary efficacy endpoint, subjects' HR status will be analyzed using a logistic regression model. The model will include the HR status (Yes vs No) as the dependent variable and treatment as the independent variable, adjusting for the stratification factors including the average of screening Hb concentrations (<8.5 vs ≥8.5 g/dL) and the *PKLR* gene mutation category (missense/missense vs missense/non-missense). The primary analysis will be based on the FAS. In the primary analysis, subjects who discontinue the study before having at least 2 Hb laboratory assessments during the Fixed Dose Period will be considered not achieving an HR. The primary result obtained from the logistic model will be the estimated odds ratio between the active arm vs the placebo arm, along with the 95% confidence interval (CI) and the 2-sided *P* value. If the 2-sided *P* value is less than the required alpha (0.05), then the null hypothesis will be rejected and a statistically significant difference will be claimed for the active arm (ie, AG-348). When the logistic model fails to converge or its maximum likelihood estimate does not exist due to quasi-complete separation, the nonparametric method (ie, Cochran-Mantel-Haenszel test [CMH]) will be used.

A sensitivity analysis based on the PPS will be conducted to evaluate the impact of missing assessments due to early study discontinuation. The number and percentage of subjects with HR will also be summarized by treatment arm.

Additional sensitivity and supportive analyses will be specified in the SAP.

12.2.4.2. Analysis of Key Secondary Endpoint

The average changes from baseline in Hb concentrations at Week 16, 20, and 24 will be analyzed and compared between the active arm and the placebo arm by the linear Mixed-Effect Model Repeat Measurement (MMRM) method. The model will include the change in Hb at each visit as

the dependent variable; treatment, visit, and treatment-by-visit interaction as fixed effects; and subject as a random effect with adjustment for the stratification factors including the average of screening Hb concentrations (<8.5 vs ≥ 8.5 g/dL) and the *PKLR* gene mutation category (missense/missense vs missense/non-missense).

The primary result obtained from the model will be the average treatment effect at Weeks 16, 20, and 24 based on the least square estimate (LS Means) from MMRM. The estimated LS Means, a 95% CI, and a 2-sided *P* value will be provided. Furthermore, the LS Means and the treatment effect at each postbaseline visit, obtained from the model, will also be provided.

12.2.4.3. Analysis of Other Secondary Efficacy Endpoints

Other Hb related secondary endpoints:

The maximum change in Hb will be summarized. The difference in the maximum change in Hb between the active vs the placebo arm, along with the 95% CI based on normal approximation, will be provided. The maximum change in Hb concentrations will be compared between the active arm and the placebo arm based on analysis of covariance (ANCOVA) controlling for the stratification factors, including the average of screening Hb concentrations and the *PKLR* gene mutation category.

The time to first HR will be analyzed using the Kaplan-Meier method. The estimated median, 25th percentile, and 75th percentile will be provided. The time to first HR will be compared between treatment arms based on a log rank test stratified by the stratification factors, including the average of screening Hb concentrations and the *PKLR* gene mutation category at a 0.05 significance level.

Other markers of hemolysis, hematopoietic activity, and indicators of clinical activity:

The changes from baseline at each visit over time in markers of hemolysis, hematopoietic activity, and indicators of clinical activities will be summarized. Additional modeling based on the MMRM method, similar to the one specified for the key secondary endpoint in Section 12.2.4.2, may be conducted and details will be provided in the SAP.

Patient-Reported Outcomes: PKDD and PKDIA

PKDD and PKDIA are currently being developed by the Sponsor, and the data from the current study will be used for their validation. The analyses used to validate these 2 instruments will be based on the final pooled blinded data conducted by the psychometric vendor. The validation details will be provided in a separate PRO validation analysis plan. Once the validation of these PROs are complete and an algorithm used to calculate their scores are finalized, the analyses of these 2 PROs will be conducted accordingly based on unblinded data.

In the final analysis of the PROs based on validated and finalized instrument, summary statistics and comparison between treatment arms will be provided as considered appropriate for the data. Additional modeling may be further conducted. Details will be provided in the SAP.

12.2.4.4. Multiplicity Adjustment

A hierarchy testing will be implemented for the primary endpoint and the key secondary endpoints. Only when the primary endpoint is significant at a 2-sided 0.05 significance level, the key secondary endpoint will be further tested.

For the other secondary efficacy endpoints, the nominal *P* values will be reported without further adjustment for multiplicity. However, these other efficacy endpoints are listed according to their clinical importance and may be considered when interpreting their nominal *P* values.

12.2.5. Safety Analysis

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

- AEs
- Clinical laboratory values
- ECGs (standard 12-lead)
- Vital signs
- PE findings
- DXA scans

Safety endpoints will be analyzed based on the SAS. Only descriptive analysis of safety will be performed (ie, no formal testing will be performed).

12.2.5.1. Adverse Events

For analysis purposes, AEs will be classified as pretreatment AEs, treatment-emergent adverse events (TEAEs), or post-treatment AEs.

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

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

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

- All TEAEs
- TEAEs by relationship
- TEAEs by maximal severity
- TEAEs leading to treatment discontinuation
- TEAEs leading to treatment interruption
- Serious TEAEs
- AESIs
- TEAEs leading to death

Summaries will be presented by Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term using frequency counts and percentages (ie, number and percentage of subjects with an event as well as total number of events). When summarizing the number and percentage of subjects with an event, subjects with multiple occurrences of the same AE or a continuing AE will be counted once, only the maximum severity level will be presented in the severity summaries, and only the worst/highest relationship level will be presented in the relationship summaries. The number of TEAEs, when each event is considered unique, will also be provided.

In addition, listings containing individual subject data for all TEAEs leading to treatment discontinuation, TEAEs leading to treatment interruption, serious AEs, AESIs, and death will be provided separately. All AEs, including pre- and post-treatment AEs, will be presented in individual subject data listings.

12.2.5.2. Clinical Laboratory Assessments

For laboratory measurements, the raw values and change from the baseline values of the continuous hematology and chemistry results will be summarized at each scheduled time point.

The number and percentage of subjects with transaminase increases of >2.5× baseline or increases to Grade ≥2 (AESI of elevated transaminase as defined in Section 11.2.6.1), will be summarized. In addition, the number and percentage of subjects who have had elevated AST/ALT (>3× ULN, >5× ULN, and >8× ULN) will be summarized.

Results of urinalysis and the serum and urine pregnancy tests will be listed in individual subject data listings only. In addition, a listing 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.

12.2.5.3. Electrocardiogram

For ECG measurements, 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, QRS, QT, and QT corrected for heart rate intervals (QTcB and QTcF), QRS duration, and heart rate.

The number and percentage of subjects will be summarized by maximum QTcB and QTcF intervals, categorized as ≤450 msec, >450 to ≤480 msec, >480 to ≤500 msec, and >500 msec, as well as maximum change from baseline, categorized as <30 msec, >30 to ≤60 msec, and >60 msec.

12.2.5.4. Vital Signs

For vital signs measurements, the raw values and change from baseline values will be summarized at each scheduled time point: systolic BP and diastolic BP (mmHg), body temperature (°C), and heart rate (beats per minute).

12.2.5.5. Physical Examination

Physical examination findings will be presented as a data listing only.

12.2.5.6. Dual-Energy X-Ray Absorption Scans

For DXA scans, the raw values of bone mineral density, T- and Z-scores, and their change from baseline values will be summarized for each scanned area and at each scheduled time point.

12.3. Interim and Independent Data Monitoring Committee Analyses

12.3.1. Interim Analysis

No interim analysis is planned.

12.3.2. Independent Data Monitoring Committee Analysis

At least 1 IDMC meeting is planned for this study. A statistician independent from the Sponsor clinical team will prepare the analysis for the IDMC. Details regarding the IDMC meeting schedules and scope of the analysis will be specified in the IDMC charter.

12.4. Sample Size and Power

12.4.1. Sample Size for the Primary Endpoint

Assuming a response rate of 35% in the active arm and 5% in the placebo arm, 76 subjects (38 per arm) are needed to have 90% power to detect a treatment effect in HR rate based on a 2-sided Fisher's Exact test with 0.05 significance level. As detailed in Section 12.2.4.1, in the primary analysis of the primary efficacy endpoint, a logistic regression model adjusting for the stratification factors, including baseline Hb and mutation type, will be used to model HR rate between the active arm and the placebo arm. Based on the logistic model, the odds ratio, along with the 95% CI and 2-sided *P* value will be provided. If the 2-sided *P* value is less than the required alpha (0.05), then the null hypothesis will be rejected and statistical significant difference will be claimed for the active arm (ie, AG-348).

The sample power calculation was based on EAST© Cytel Inc. Version 6.4.

12.4.2. Power for the Key Secondary Endpoint

Assuming a standard deviation of 1.5, a sample size of 76 subjects (38 per arm) has 80% power to detect a difference of 1.4 in the average change in Hb at Weeks 16, 20, and 24, based on the simple 2-sample t-test. As specified in Section 12.2.4.2, the final analysis of the key secondary endpoint will be based on MMRM. The average treatment effect at Weeks 16, 20, and 24 will be compared between active and placebo arms based on the LS Means from MMRM at a 2-sided 0.05 significance level. A hierarchy testing will be implemented for the primary and the key secondary endpoint.

13. ADMINISTRATIVE REQUIREMENTS

13.1. Good Clinical Practices

The study will be conducted in accordance with the International Council for Harmonisation (ICH) for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s). The Investigator will be thoroughly familiar with the appropriate use of the study treatment as described in the protocol and the IB. 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.

The Investigator must obtain IRB/IEC 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/IEC 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/IEC approval has been obtained. The protocol, IB, ICF, advertisements (if applicable), written information given to the subjects, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB/IEC.

The IRB/IEC 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/IEC 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 treatment 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 the Sponsor's 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/IEC approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to subjects. The IRB/IEC 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/IEC. 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 Medical Monitor (or Medical Director), 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

All data for the subjects recruited for the trial will be entered onto the eCRFs via an EDC system provided by the Sponsor, or designee. Only authorized staff may enter data onto the eCRFs. If an entry error is made, the corrections to the eCRFs will be made according to eCRF guidelines by an authorized member of the site staff.

Electronic case report forms will be checked for correctness against source document data by the Sponsor's monitor. If any entries into the eCRF are incorrect or incomplete, the monitor will ask the Investigator or the study site staff to make appropriate corrections, and the corrected eCRF will again be reviewed for completeness and consistency. Any discrepancies will be noted in the eCRF system by means of electronic data queries. Authorized site staff will be asked to respond to all electronic queries according to the eCRF guidelines.

13.7. Source Documentation and Electronic 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/eCRF. 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 for a subject's examination, treatment, or any other study procedure. Any outstanding entries must be completed 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 the Sponsor's designee. Monitoring will be performed 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 treatment and other study materials should be destroyed or returned to the Sponsor, or designee, after the study has been completed, as directed by the Sponsor.

Regulatory authorities, the IRB/IEC, 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 only to conduct the study and not to use it for any other purpose without explicit consent from the Sponsor.

It is understood that there is an obligation on the Investigator's part to provide the Sponsor with the complete data obtained during the study. Such information will be used in the clinical development of AG-348 and may be disclosed to regulatory authorities, other Investigators, corporate partners, or consultants, as required.

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