CLINICAL STUDY PROTOCOL AG348-C-007

AN OPEN-LABEL STUDY TO EVALUATE THE EFFICACY AND SAFETY OF AG-348 IN REGULARLY TRANSFUSED ADULT SUBJECTS WITH PYRUVATE KINASE (PK) 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: 21 March 2019 | 09:53 EDT
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[redacted], MD
[redacted]
[redacted]
Agios Pharmaceuticals, Inc.

21-Mar-2019 | 9:53 AM EDT
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:
An Open-Label Study to Evaluate the Efficacy and Safety of AG-348 in Regularly Transfused Adult Subjects With Pyruvate Kinase (PK) Deficiency

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

Phase of Development: 3

Objectives:

Primary: The primary objective of the study is to evaluate the efficacy of treatment with AG-348, as assessed by the reduction in transfusion burden.

Secondary: The secondary objective of this study is to evaluate the safety of treatment with AG-348.

Exploratory:

- To determine the effect of AG-348 on markers of hemolysis, erythropoietic activity, and other indicators of clinical activity
- To determine the effect of AG-348 on markers of iron metabolism and indicators of iron overload
- To determine the effect of AG-348 on health-related quality of life (HRQoL), as determined using patient-reported outcomes
- To evaluate the pharmacokinetics of AG-348 after oral administration
- To evaluate the relationship of AG-348 pharmacokinetics to indicators of clinical activity
- To evaluate the change in the levels of red blood cell (RBC)-specific form of pyruvate kinase (PKR) protein in whole blood

Methodology:

Study AG348-C-007 is a 2-part, multicenter, open-label, Phase 3 study consisting of a Dose Optimization Period (Part 1) followed by a Fixed-Dose Period (Part 2). This study will evaluate the efficacy and safety of treatment with AG-348 in a minimum of 20, with up to 40, adult subjects with pyruvate kinase deficiency (PK deficiency) who are regularly receiving blood transfusions.

Prior to Part 1 of the study, there will be an 8-week Screening Period in which a subject's complete transfusion history from 52 weeks prior to signing the Informed Consent Form (ICF) will be collected and recorded on the source documentation and electronic case report form (eCRF).

The transfusion history information will be used as follows:

- To determine whether the subject meets the study criteria for regular transfusion frequency
- To calculate the Mean Transfusion Frequency (MTF)
- To calculate the Individual Transfusion Trigger (TT), which is the mean (±0.5 g/dL or ±0.31 mmol/L) of a subject's collected historical pretransfusion hemoglobin (Hb) concentrations. For each transfusion, only the Hb concentration within 1 week prior to and closest to a transfusion will be included in this calculation.

- To calculate the Mean Number of Blood Units (MNU) transfused per transfusion
- To function as historical control data to be used in the analysis

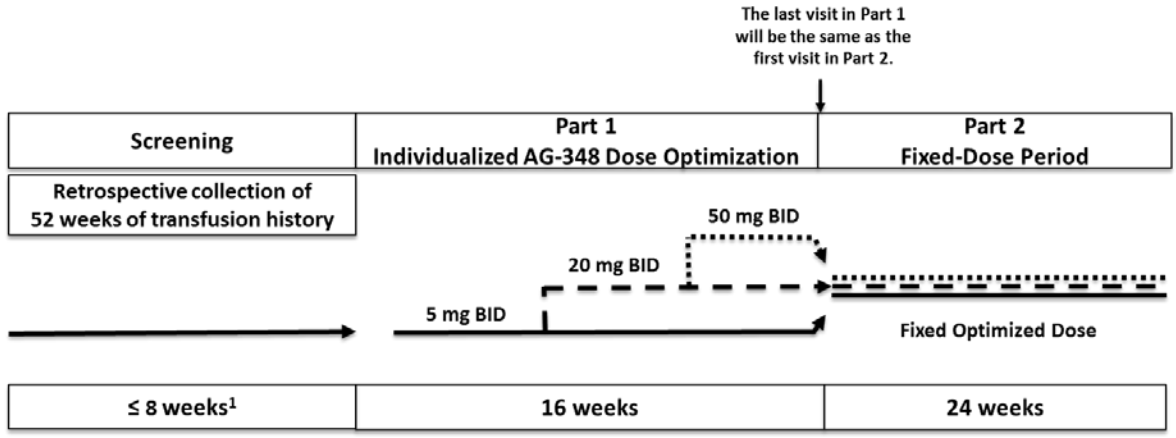
All subjects will receive AG-348 in Parts 1 and 2 of the study. During Part 1 of the study, all subjects will start on a dose of 5 mg AG-348 administered twice daily (BID). Over the course of Part 1, each subject will undergo intrasubject dose optimization. Each subject's dose level of AG-348 may be increased 2 times beyond the starting dose of 5 mg BID (ie, from 5 to 20 mg BID and from 20 to 50 mg BID). In the Fixed-Dose Period of the study (Part 2), a subject will receive AG-348 at his/her optimized dose with no planned adjustments (ie, as a fixed dose) for a fixed period of 24 weeks.

All subjects who remain on study during Part 2 through the Week 24 Visit may be eligible for an open-label extension study with AG-348. Subjects who continue on into an extension study will not be required to attend the Follow-up Visit. Subjects who continue the study through the Part 2 Week 24 Visit on study drug but do not continue on into an extension study will attend the Follow-up Visit 28±4 days after the last dose of study drug. Subjects who discontinue the study prior to the Part 2 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 drug, whichever is later.

All subjects who discontinue study drug at any time during the study should undergo a dose taper as described in this protocol, unless an emergency situation justifies discontinuing the study drug abruptly. Whether the dose taper is performed or not, subjects discontinuing AG-348 should be monitored for signs of hemolysis and worsening of anemia.

An overview of the study design is shown below.

Figure: Overview of Design for Study AG348-C-007



Abbreviation: BID = twice daily.

[1] Screening may be extended beyond 8 weeks if there is a delay in obtaining a subject's complete transfusion history or to ensure that the first dose of study drug can be administered 2-7 days after the most recent transfusion, upon approval by the Medical Monitor (or designee).

Number of Subjects (Planned):

A minimum of 20, with up to 40, subjects

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 study central genotyping laboratory.
4. Have a history of a minimum of 6 transfusion episodes in the 52-week period prior to date of informed consent as documented in the transfusion history of the subject, which reflects the subject's typical transfusion burden.
5. Have complete records of transfusion history, defined as having the following available for the 52 weeks prior to the date of informed consent: (1) **all** the transfusion dates, (2) the number of blood units transfused for **all** the transfusions, and (3) Hb concentrations within 1 week prior to transfusion for at least 80% of the transfusions.
6. Have received at least 0.8 mg oral folic acid daily for at least 21 days prior to the first dose of study drug, to be continued daily during study participation.
7. Have adequate organ function, as defined by:
   a. Serum aspartate aminotransferase (AST) $\leq 2.5 \times$ upper limit of normal (ULN) (unless the increased AST is assessed by the Investigator as due to hemolysis and/or hepatic iron deposition) and alanine aminotransferase (ALT) $\leq 2.5 \times$ 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 (ANC) $\geq 1.0 \times 10^9$/L.
   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.
   f. Activated partial thromboplastin time (aPTT) and international normalized ratio (INR) $\leq 1.25 \times$ 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 drug for

women and 90 days following the last dose of study drug 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 drug.

10. Be willing to comply with all study procedures, in particular the Individual TT (calculated based on 52 weeks of transfusion history), 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 study 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 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. 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 RBC disorders).
    i. Positive test for hepatitis B surface antigen (HBsAg) 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-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 [NCI CTCAE]) within 2 months prior to the first dose of study drug.

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 history of transfusions occurring on average more frequently than once every 3 weeks during the 52 weeks prior to signing informed consent.
4. Have a splenectomy scheduled during the study drug period or have undergone splenectomy within 12 months prior to signing informed consent.
5. 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.
6. Have exposure to any investigational drug, device, or procedure within 3 months prior to the first dose of study drug.
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 drug.
11. Are currently receiving hematopoietic stimulating agents (eg, erythropoietins [EPOs], 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 drug.
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 drug.

Investigational Product, Dosage and Mode of Administration:

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

Duration of Treatment:

The maximum total duration that a subject could receive AG-348 in this study is 40 weeks (not including the time required to taper study drug).

The duration of AG-348 treatment at each dose level and the overall duration of the Dose Optimization Period (Part 1) of the study will be 16 weeks. The subject's last visit in Part 1 will be the first visit in the Fixed-Dose Period (Part 2). In Part 2 of the study, subjects will receive their optimized dose of AG-348 for 24 weeks.

Reference Therapy, Dosage and Mode of Administration:

Not applicable.

Criteria for Evaluation:

Study Endpoints:

Primary Endpoint:

The primary endpoint of this study is the proportion of subjects who achieve a reduction in transfusion burden, defined as a ≥33% reduction in the number of RBC units transfused during the 24 weeks of Part 2 compared with the historical transfusion burden standardized to 24 weeks (Standardized Control Period).

Secondary Endpoints:

The secondary endpoints of the study are the following:

- Annualized total number of RBC units transfused during the study (both Part 1 and Part 2) compared with the historical transfusion burden
- Number of transfusion episodes during Part 2 compared with the Standardized Control Period
- Proportion of subjects who become transfusion-free, defined as 0 transfusions administered during Part 2
- Proportion of subjects who achieve Hb concentrations in the normal range at least once, 8 weeks or more after a transfusion in Part 2
- Safety endpoints, including the type, incidence, severity, and relationship to treatment of adverse events (AEs) and serious adverse events (SAEs), and AEs leading to treatment dose reduction, treatment interruption, and treatment discontinuation
- Laboratory tests over time (eg, serum chemistry, liver function tests [LFTs], hematology, coagulation, lipids, sex steroids, urinalysis), physical examination findings, dual-energy X-ray absorptiometry (DXA) scans (hip and lumbar spine), vital signs, and 12-lead electrocardiogram (ECG) data

Exploratory Endpoints:

The exploratory endpoints of the study are the following:

- Change from baseline in the following markers of hemolysis: bilirubin, lactate dehydrogenase (LDH), and haptoglobin levels
- Change from baseline in markers of erythropoietic activity
- Change from baseline in markers of iron metabolism and indicators of iron overload
- Change from baseline over time in HRQoL scores (ie, Pyruvate Kinase Deficiency Impact Assessment [PKDIA], Pyruvate Kinase Deficiency Diary [PKDD], EuroQol-5D-5L [EQ-5D-5L])

- Characterization of pharmacokinetic profile (drug concentrations over time) and determination of pharmacokinetic parameters of AG-348 (eg, area under the plasma concentration × time curve [AUC], maximum [peak] concentration [C_{max}], and others as applicable) in Part 2
- Exposure-response (or pharmacokinetic-pharmacodynamic) relationship between relevant pharmacokinetic parameters and endpoints that are indicators of clinical activity
- Changes in total PKR protein levels

Statistical Methods:

Sample Size:

Due to the rarity of the disease and the small patient population, the sample size is largely driven by feasibility. Given the feasibility results and uncertainty of the screen failure rate, the study will enroll a minimum of 20, with up to 40, subjects. With a sample size of 20, the power of the study is limited, that is, the power will be 58% to detect a response rate of 30% compared to a null rate of 10% with an exact test at 2-sided 0.05 significance level. There will be 75% power to detect a large response rate of 35%, compared to the null of 10%. When the sample size is 40, the power will be at least 90%, even if the target response rate is 30%.

Analysis Sets:

Two analysis sets will be defined for evaluation of the study endpoints:

- The Full Analysis Set (FAS) is defined as all subjects who received at least 1 dose of study drug. The FAS will be used for primary efficacy analyses and safety analyses, unless specified otherwise.
- The Per Protocol Set (PPS) is defined as a subset of the FAS including subjects who have completed Part 2. The PPS will be used for sensitivity analysis for the primary endpoint.

Statistical Methods:

Statistical analysis details will be provided in the statistical analysis plan (SAP), which will be finalized before the database lock.

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

Efficacy Analysis

For the primary analysis of the primary endpoint, the number and proportion of subjects who achieve a transfusion reduction response, defined as ≥33% reduction in the number of RBC units transfused during the 24 weeks in Part 2 compared to the historical transfusion burden standardized to 24 weeks (Standardized Control Period), along with its 95% CI based on the exact binomial distribution, will be provided based on the FAS. Subjects who discontinue the study before completing 12 weeks of treatment in Part 2 will be considered nonresponders. No formal hypothesis test will be conducted. The focus will be given to descriptive statistics, including both the point estimate and the CIs.

The similar set of analyses will be repeated based on PPS, to further evaluate the impact of early discontinuation on the efficacy results.

For the analysis of the secondary efficacy endpoints of annualized total number of RBC units transfused during the study and number of transfusion episodes during Part 2, their change and percentage change from the corresponding normalized historical transfusion burden will be summarized using descriptive statistics.

Additional analyses include:

1. Number and percentage of subjects who are transfusion-free in the Fixed-Dose Period (Part 2), along with its 95% CI based on the exact binomial distribution, will be provided.

2. Number and percentage of subjects who achieve Hb concentrations in the normal range at least once, 8 weeks or more after a transfusion in Part 2, along with its 95% CI based on the exact binomial distribution, will be provided.

Additional sensitivity analysis, supportive analysis, and analyses for exploratory endpoints and possibly additional endpoints will be described in the SAP.

The overall safety profile of study drug will be assessed based on summary of treatment-emergent AEs, clinical laboratory values, ECGs, vital signs, physical examination findings, and DXA scans, based on the FAS. Only descriptive analysis will be performed (ie, no formal testing will be performed).

Interim Analysis and Independent Data Monitoring Committee:

No interim analysis is planned, but interim analyses may take place at any time during the study, if warranted, by the ongoing data and/or deemed necessary by the internal Agios team. No independent data monitoring committee is planned for this study.

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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
ALC	Absolute lymphocyte count
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
AUC	Area under the 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
BMD	Bone mineral density
BP	Blood pressure
BUN	Blood urea nitrogen
CL/F	Apparent total plasma clearance following oral (extravascular) dosing
C_{max}	Maximum (peak) concentration
CLp	Plasma clearance
CO_2	Carbon dioxide
CrCL	Calculated creatinine clearance
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

Abbreviation	Definition
EPO	Erythropoietin
EQ-5D-5L	EuroQol-5D-5L
FAS	Full Analysis Set
FSH	Follicle-stimulating hormone
GCP(s)	Good Clinical Practice(s)
GDT	Gradual Dose Taper
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
HR	Hemoglobin response
HRQoL	Health-related quality of life
IB	Investigator's Brochure
IC_{50}	Half-maximal inhibitory concentration
ICF	Informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IMP	Investigational medicinal product
INR	International normalized ratio
IRB	Institutional Review Board
LDH	Lactate dehydrogenase
LDL-C	Low-density lipoprotein cholesterol
LIC	Liver iron concentration
MAD	Multiple-ascending dose
max	Maximum
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume

Abbreviation	Definition
min	Minimum
MNU	Mean Number of Blood Units
MRI	Magnetic resonance imaging
MTF	Mean Transfusion Frequency
n	Number of subjects
NCI	National Cancer Institute
NHS	Natural History Study
NRBC	Nucleated red blood cell count
NTBI	Non-transferrin bound iron
PD	Pharmacodynamic(s)
PEP	Phosphoenolpyruvate
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
PTH	Parathormone
QD	Once daily
QOD	Every other day
QTcB	Heart rate-corrected QT interval by Bazett's method
QTcF	Heart rate-corrected QT interval by Fridericia's method
QnW	Every n weeks
RBC	Red blood cell
RDW	Red cell distribution width
RT-PCR	Reverse transcriptase-polymerase chain reaction
SAD	Single-ascending dose
SAE	Serious adverse event

Abbreviation	Definition
SAP	Statistical analysis plan
SD	Standard deviation
$t_{½}$	Terminal half-life
T4	Thyroxine
TEAE	Treatment-emergent adverse event
TIBC	Total iron-binding capacity
T_{max}	Time to maximum (peak) concentration
T_{last}	Time of last measurable concentration
TSH	Thyroid-stimulating hormone
TRR	Transfusion reduction response
TT	Transfusion Trigger
ULN	Upper limit of normal
WBC	White blood cell
WT	Wild-type
Vss	Volume of distribution at steady state
Vz/F	Volume of distribution during the terminal elimination phase following oral (extravascular) dosing

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

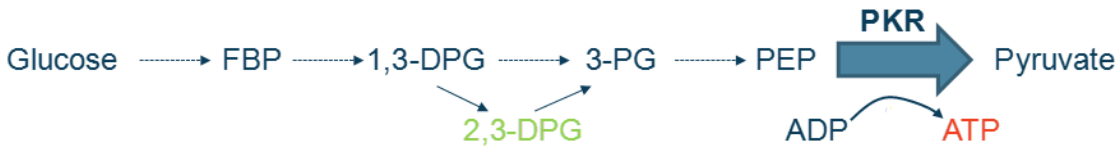
Epidemiological data for PK deficiency are scarce; however, the current estimated diagnosed prevalence of patients with PK deficiency in the United States (US) and European Union 5 (EU5) (ie, France, Germany, Italy, Spain, and the United Kingdom) is approximately 2,400 cases (Carey et al, 2000; de Medicis 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 to 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 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 nondrug 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) (ClinicalTrials.gov, 2017)—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 identified only 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.

5.1.3.1. Transfusion Frequency

There are no guidelines for transfusion management in patients with PK deficiency. Most adults with the disease have undergone splenectomy 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."

A minority of adult patients with PK deficiency still require regular transfusions after splenectomy. Some adult patients who have not undergone splenectomy also require regular transfusions. 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. For patients transfused infrequently, it can be difficult to determine retrospectively whether a patient is transfused mainly for cause or requires infrequent, but still regular transfusions. Experts have indicated patients receiving fewer than 6 transfusions per year (every 2 months on average over a 1-year period) are unlikely to require the transfusions to maintain hemoglobin (Hb) at an acceptable level.

Analysis of the NHS study, which has data for a total of 255 subjects as of 28 April 2017, identified a maximum of 16 adult subjects (out of 98 non-Amish subjects) who reported receiving at least 6 transfusions in the year prior to enrollment (Grace et al, 2018). This severely limits the type of clinical trials that could be performed in this population.

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 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 data 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 is being used in several of the ongoing studies, was 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.

Please 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 current 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-007, will be conducted in adult subjects with PK deficiency who are regularly receiving transfusions, while a separate study, AG348-C-006, will be conducted in adult subjects with PK deficiency who are not 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 require regular transfusions to maintain their Hb concentration at a level considered acceptable. Iron overload, an unavoidable complication of chronic hemolytic anemia, which is usually incompletely controlled by iron chelation therapy, is made worse by splenectomy and frequent transfusions. The consequences of transfusion-related iron overload if inadequately treated include liver dysfunction, cardiomyopathy (which may be fatal), and endocrinopathy, including diabetes, hypogonadism, and infertility. All this contributes to a clear unmet medical need for the regularly transfused population of patients with PK deficiency.

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. This clinical study will determine whether AG-348 provides benefit to the regularly transfused patient population by decreasing their need for regular transfusions.

5.3.1. Justification of Study Design

Given the rarity of patients with PK deficiency in general and the extreme rarity of regularly transfused patients with PK deficiency (less than 20% of the non-Amish adult population with PK deficiency according to the NHS), it is not possible to conduct an adequately powered randomized controlled study. Therefore, Study AG348-C-007 has a single-arm design in which each subject's own historical transfusion data will be used as their individual control. Efficacy will be measured by the change in transfusion burden when subjects are on an individually optimized dose of AG-348 compared with each subject's historical transfusion burden. Subjects will be treated on study for up to 40 weeks, including up to 16 weeks for dose optimization and 24 weeks on their individual optimized dose. This length of time was selected in order to have a reasonable estimate of the transfusion frequency on a stable dose of AG-348 in subjects whose historical transfusion frequency could range from every 3 weeks to every 8 weeks. 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 5.3.1.3 for further justification regarding specific aspects of the study design.

5.3.1.1. Justification Related to Sample Size

As previously mentioned (Section 5.3.1), it is estimated that less than 20% of the non-Amish adult population with PK deficiency is regularly transfused. A feasibility assessment and outreach effort to evaluate the accessibility of a subject population meeting the criteria for this proposed Phase 3 study have thus far identified 36 potential subjects in 22 sites within 13 countries. Although there is uncertainty with the screen failure rate, a number of these patients may be ineligible for the trial, for a variety of reasons.

5.3.1.2. Justification of Using Individual Transfusion History Data as Comparator to Assess Efficacy of AG-348

Each subject's own historical transfusion data will be used as his/her individual control to assess efficacy (ie, reduction in transfusion need while the subject is receiving AG-348). Using

historical controls rather than a placebo control group is an established method in rare diseases (Moscicki and Tandon, 2017). More specifically, individual patients' transfusion histories have been used as a comparator in multiple studies conducted in frequently transfused patients with various chronic anemias (eg, beta-thalassemia, paroxysmal nocturnal hemoglobinuria). It is especially valuable in heterogeneous patient populations, such as regularly transfused patients with PK deficiency where the absence of transfusion guidelines translates to patients with apparently similar degrees of anemia potentially being treated differently.

5.3.1.3. Justification of Duration for Collection of Historical Data and Duration on Optimized Dose

This study includes a collection of 52 weeks of historical transfusion data prior to screening. The duration of 52 weeks was selected in order to limit the impact of any ad hoc transfusion administered for an acute hemolytic episode on the estimation of each subject's basal transfusion need.

The subjects enrolled into this study will have a transfusion frequency at a minimum of around every 8 weeks. Therefore, a 16-week duration for dose optimization followed by a 24-week duration on a stable dose should ensure an appropriate estimate of the transfusion rate while subjects are receiving AG-348.

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 conducted in subjects who do not require regular transfusions. 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 (5 mg BID) 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 in Study AG348-C-006, which will enroll subjects with PK deficiency who

are not regularly transfused. Patients who are regularly transfused and those who do not need regular transfusions represent the same disease caused by the same genetic deficiency in the *PKLR* gene; therefore, the same doses have been selected for this current study.

5.3.2.1. Justification of Individual Dose Optimization

Preliminary results of the DRIVE-PK study indicate that multiple 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 resolutions of most of these AEs and the maintenance of a satisfactory but not excessive level of Hb where applicable.

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. For this reason, all subjects will start at a low dose level (5 mg BID) with 2 sequential steps for dose level increases (ie, from 5 to 20 mg BID and from 20 to 50 mg BID), depending on safety (see Section 7.1.3 for dose optimization guidelines).

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, as assessed by the reduction in transfusion burden.

6.1.2. Secondary Objectives

The secondary objective of this study is to evaluate the safety of treatment with AG-348.

6.1.3. Exploratory Objectives

The exploratory objectives of the study are the following:

- To determine the effect of AG-348 on markers of hemolysis, erythropoietic activity, and other indicators of clinical activity
- To determine the effect of AG-348 on markers of iron metabolism and indicators of iron overload
- To determine the effect of AG-348 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 of AG-348 pharmacokinetics to indicators of clinical activity
- To evaluate the change in the levels of PKR protein in whole blood

6.2. Endpoints

6.2.1. Primary Endpoint

The primary endpoint of this study is the proportion of subjects who achieve a reduction in transfusion burden, defined as a ≥33% reduction in the number of RBC units transfused during the 24 weeks of Part 2 compared with the historical transfusion burden standardized to 24 weeks (Standardized Control Period).

6.2.2. Secondary Endpoints

The secondary endpoints of the study are the following:

- Annualized total number of RBC units transfused during the study (both Part 1 and Part 2) compared with the historical transfusion burden
- Number of transfusion episodes during Part 2 compared with the Standardized Control Period
- Proportion of subjects who become transfusion-free, defined as 0 transfusions administered during Part 2

- Proportion of subjects who achieve Hb concentrations in the normal range at least once, 8 weeks or more after a transfusion in Part 2
- Safety endpoints, including the type, incidence, severity, and relationship to treatment of AEs and serious adverse events (SAEs), and AEs leading to treatment dose reduction, treatment interruption, and treatment discontinuation
- Laboratory tests over time (eg, serum chemistry, liver function tests [LFTs], hematology, coagulation, lipids, sex steroids, urinalysis), physical examination (PE) findings, dual-energy X-ray absorptiometry (DXA) scans (hip and lumbar spine), vital signs, and 12-lead electrocardiogram (ECG) data

6.2.3. Exploratory Endpoints

The exploratory endpoints of the study are the following:

- Change from baseline in the following markers of hemolysis: bilirubin, lactate dehydrogenase (LDH), and haptoglobin levels
- Change from baseline in markers of erythropoietic activity
- Change from baseline in markers of iron metabolism and indicators of iron overload
- Change from baseline over time in HRQoL scores (ie, Pyruvate Kinase Deficiency Impact Assessment [PKDIA], Pyruvate Kinase Deficiency Diary [PKDD], EuroQol-5D-5L [EQ-5D-5L])
- Characterization of pharmacokinetic profile (drug concentrations over time) and determination of pharmacokinetic parameters of AG-348 (eg, AUC, C_{max}, and others as applicable) in Part 2
- Exposure-response (or pharmacokinetic-PD) relationship between relevant pharmacokinetic parameters and endpoints that are indicators of clinical activity
- Change from baseline in PKR protein levels

7. INVESTIGATIONAL PLAN

7.1. Study Design

7.1.1. Overview of Study Design

This is a 2-part, multicenter, open-label, Phase 3 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 treatment with AG-348 in a minimum of 20, with up to 40, adult subjects with PK deficiency who are regularly receiving blood transfusions.

Prior to Part 1 of the study, there will be an 8-week Screening Period in which a subject's complete transfusion history from 52 weeks prior to signing the Informed Consent Form (ICF) will be documented. This information, taken from the subject's medical record and entered into the electronic case report form (eCRF), will be used to inform study eligibility and analyze data.

All subjects will receive AG-348 in Parts 1 and 2 of the study. During Part 1 of the study, all subjects will start on a dose of 5 mg AG-348 administered BID. Over the course of Part 1 of the study each subject will undergo intrasubject dose optimization per Section 7.1.3. Each subject's dose level of AG-348 may be increased 2 times beyond the starting dose of 5 mg BID (ie, from 5 to 20 mg BID and from 20 to 50 mg BID). In the Fixed-Dose Period of the study (Part 2), a subject will receive AG-348 at his/her optimized dose with no planned adjustments (ie, as a fixed dose) for a fixed period of 24 weeks.

All subjects who remain on study during Part 2 through the Week 24 Visit may be eligible for an open-label extension study with AG-348. Subjects who continue on into an extension study will not be required to attend the Follow-up Visit. Subjects who continue the study through the Part 2 Week 24 Visit on study drug but do not continue on into an extension study will attend the Follow-up Visit 28±4 days after the last dose of study drug.

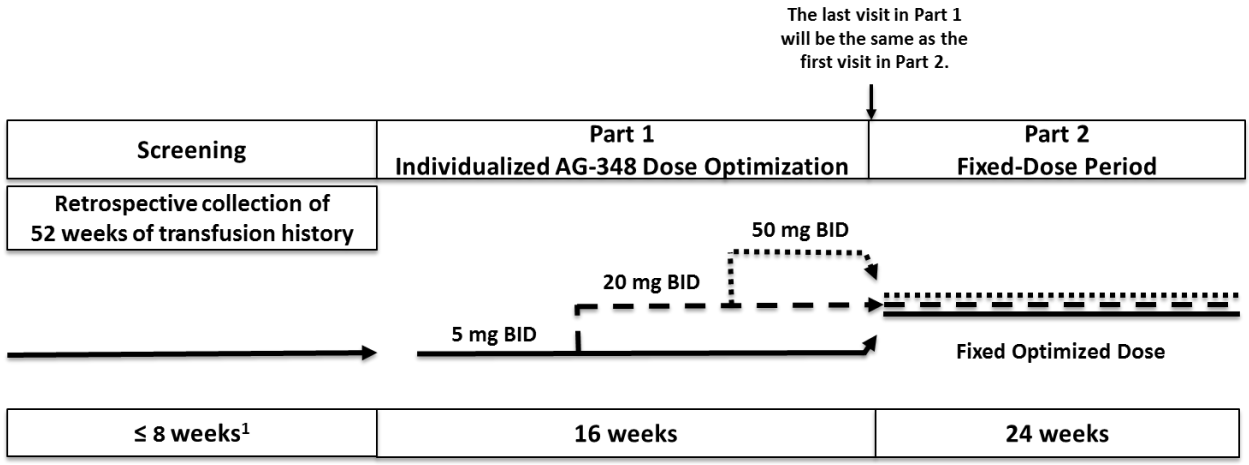
Subjects who discontinue the study prior to the Part 2 Week 24 Visit should attend the End of Study Visit 28±4 days after the last study visit that the subject attended or 28±4 days after the last dose of study drug, whichever is later.

All subjects who discontinue study drug at any time during the study should undergo a dose taper as described in Section 9.3, unless an emergency situation justifies discontinuing the study drug abruptly. Whether the dose taper is performed or not, subjects discontinuing AG-348 should be monitored for signs of hemolysis and worsening of anemia.

The maximum total duration that a subject can receive AG-348 in this study is 40 weeks (not including the dose taper).

The Screening Period, Part 1, Part 2, and follow-up of the study are described in more detail in Section 7.1.2, Section 7.1.3, Section 7.1.4, and Section 7.1.5, respectively. Details for dose reductions or interruptions required for reasons of safety are in Section 9.3.

Figure 2: Overview of Design for Study AG348-C-007



Abbreviation: BID = twice daily.
[1] Screening may be extended beyond 8 weeks if there is a delay in obtaining a subject's complete transfusion history or to ensure that the first dose of study drug can be administered 2-7 days after the most recent transfusion, upon approval by the Medical Monitor (or designee).

7.1.2. Screening Period

Screening will occur over an 8-week period after a subject provides written informed consent. During the Screening Period, each subject's transfusion history in the past 52 weeks will be collected. The Investigator will determine whether each subject meets all of the inclusion criteria and none of the exclusion criteria. Eligibility of each subject will be confirmed by the Medical Monitor (or designee). A subject will not be considered enrolled (and cannot receive his/her first dose of study drug) until eligibility is confirmed.

7.1.2.1. Transfusion History

As described in Section 5.3.1.2, an established method of obtaining historical control data will be used to collect subjects' transfusion history at Screening. For each subject, the following transfusion history information for the 52 weeks prior to signing the ICF will be collected and recorded on the source documentation and eCRF:

- Dates of all transfusions
- Pre-transfusion Hb concentrations (Hb concentrations within 1 week prior to each transfusion episode) for at least 80% of the transfusions
- For each transfusion, number of blood units transfused
- When available, hematocrit (HCT) and volume of transfused blood units

The transfusion history information will be used as follows:

- To determine whether the subject meets the study criteria for regular transfusion frequency (see Inclusion Criterion #4 [Section 8.1] and Exclusion Criterion #3 [Section 8.2])
- To calculate the Mean Transfusion Frequency (MTF)

- To calculate the Individual Transfusion Trigger (TT), which is the mean (±0.5 g/dL or ±0.31 mmol/L) of a subject's collected historical pretransfusion Hb concentrations. For each transfusion, only the Hb concentration within 1 week prior to and closest to a transfusion will be included in this calculation.
- To calculate the Mean Number of Blood Units (MNU) transfused per transfusion
- To function as historical control data to be used in the analysis

The MTF (transfusion every X week[s]) will be calculated by dividing 52 weeks by the total number of transfusions received in the 52-week period prior to informed consent. For example, a subject who received 13 transfusions in the 52-week period would have an MTF of every 4 weeks (52 weeks/13 transfusions = transfusion every 4 weeks). Fractional numbers will be rounded to the closest integer (eg, 52 weeks/17 transfusions = 3.06 weeks rounded to every 3 weeks). All transfusions will be included in this calculation, and no attempt will be made to distinguish transfusions administered for cause (eg, acute infection) and transfusions administered systematically.

The Individual TT will be the sum of the Hb concentrations within 1 week prior to and closest to each transfusion (when available) for the transfusions in the 52-week period prior to informed consent divided by the number of such available Hb concentrations during that period, calculated to the first decimal point, if expressed in g/dL, or to the second decimal point, if expressed in mmol/L. A margin of ±0.5 g/dL (or ±0.31 mmol/L) will be attached to this value. For example, a subject who had 7 pre-transfusion Hb concentrations in the 52-week period prior to informed consent would have an Individual TT of 7.0±0.5 g/dL based on the following pre-transfusion Hb concentrations (g/dL): 7.0, 7.2, 6.8, 6.8, 6.7, 7.2, and 7.1.

The MNU will be the sum of the number of units transfused at each transfusion during the 52-week period prior to informed consent divided by the number of transfusions during this period. Fractional numbers will be rounded to the closest integer (eg, 13 units/6 transfusions = 2.2 units rounded to 2 units; 25 units/10 transfusions = 2.5 units rounded to 3 units).

A subject's Screening Period duration may be extended beyond 8 weeks upon the Medical Monitor's (or designee's) approval if there is a delay in obtaining a subject's complete transfusion history, or the subject is projected to need a transfusion within 1 week of his/her scheduled first dose to ensure that treatment with AG-348 is always started 2-7 days after a subject's most recent transfusion in the Screening Period (referred to as Transfusion 0).

7.1.3. Part 1: Dose Optimization Period

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 AG-348, with 2 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 drug 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 10.14.1, Table 4).

Subjects will be assessed for safety and efficacy during Part 1 to determine if their dose should be increased, maintained at the current level, or decreased. The Investigator will be expected to be vigilant about monitoring each subject's Hb concentrations throughout the study in order to evaluate the need for a transfusion (by comparing the subject's Hb concentration with his/her Individual TT). Investigators will assess the overall status of the subject and the subject's tolerability to make a decision on study drug dose. The following rules apply:

- At the Week 4 Visit, study drug dose should be increased to the next dose level (ie, from 5 mg BID to 20 mg BID) if the subject has not experienced any study drug-related safety issues.
- The dose should be increased either at the Week 10 Visit if the subject has reached his/her Individual TT (and has been transfused) between Week 8 and Week 10, or when the subject reaches the Week 12 Visit (without having reached his/her Individual TT between Week 8 and Week 12). In either case, the study drug dose should be increased to the next dose level if the subject has not experienced any study drug-related safety issues.

If the dose was not increased at the Week 4 Visit, and the Investigator is considering increasing the dose at a subsequent visit, the Investigator should contact the Medical Monitor (or designee) for guidance.

The study drug dose should not be increased at any of these visits if Hb concentration from the previous visits (per the central laboratory analysis) is ≥14.5 g/dL (9.0 mmol/L) in males or ≥13.0 g/dL (8.07 mmol/L) in females.

If the Investigator deems it necessary to reduce the study drug dose for safety reasons, the subject's dose may be reduced to 1 of the 2 available lower dose levels (ie, 5 mg BID or 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 Medical Monitor (or designee).

Dose escalation or re-introduction should be avoided after the Week 12 Visit but may be permitted after discussion with the Medical Monitor (or designee) for subjects who had their dose reduced or suspended for reasons related to safety.

If questions arise about whether the dose level of a given subject should be increased, maintained, or decreased, the site is advised to contact the Medical Monitor (or designee) for discussion. **At any time during the study, the Investigator can reduce the subject's dose or interrupt study drug for reasons related to safety (as described in Section 9.3).**

Assessments and timing details for Part 1 are outlined in the Schedule of Assessments (Table 4).

7.1.3.1. Transfusions During Part 1

The scheduled hematology assessments in Part 1 will be used to determine whether a subject has reached his/her Individual TT, in which case the subject should be transfused with his/her MNU of packed RBCs.

Subjects, in particular those with a historical MTF of every 3 or 4 weeks, may need additional hematology assessments (Hb concentration) if the subject's Individual TT has not been reached at a scheduled visit. Blood sampling for these additional assessments (unscheduled visits) should

be analyzed by the central laboratory but, in extenuating situations, may be conducted and analyzed by a local laboratory or physician's office local to the subject, as feasible and allowed by Investigators and local regulations.

All on-study transfusion data will be recorded as noted in Section 9.4.

7.1.4. Part 2: Fixed-Dose Period

In Part 2 of the study, subjects will receive their optimized dose of AG-348 for 24 weeks. Dose escalation or re-introduction should be avoided during Part 2 but may be permitted after discussion with the Medical Monitor (or designee) for subjects who had their dose reduced or suspended for reasons related to safety.

Assessments and timing details for Part 2 are outlined in the Schedule of Assessments (Table 5).

7.1.4.1. Transfusions During Part 2

Subjects should continue to be transfused when their Hb concentration decreases to their Individual TT. Some subjects may need additional hematology assessments (Hb concentration), for example, if the subject's Individual TT has not been reached at a scheduled visit. Blood sampling for these additional assessments (unscheduled visits) should be analyzed by the central laboratory but, in extenuating situations, may be conducted and analyzed by a local laboratory or physician's office local to the subject, as feasible and allowed by Investigators and local regulations.

All on-study transfusion data will be recorded as noted in Section 9.4.

7.1.5. Discontinuation and Follow-up

Subjects who discontinue the study prior to the Part 2 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 drug, whichever is later. This visit will be identical to the Part 2 Week 24 Visit with these exceptions: no study drug will be dispensed at the End of Study Visit, blood sampling for pharmacokinetic profile will not be drawn, PKR protein will not be assessed, and if the subject has not already done so, they should return their eDiary and study drug.

Subjects who continue study drug through Part 2 Week 24 should continue taking study drug at least through the morning dose of the Part 2 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 with AG-348.

- Subjects who continue study drug through the Part 2 Week 24 Visit but do not continue on into an extension study should undergo a dose taper and then attend the Follow-up Visit 28±4 days after the last dose of study drug.
- Subjects who continue on into an extension study will not be required to attend the Follow-up Visit.
- Subjects who initiate or are undergoing a dose taper for a reason related to safety and for whom participation in an extension study remains undetermined should perform the taper as detailed in Section 9.3. Transition to an extension study should then be discussed with the Medical Monitor (or designee).

All subjects who discontinue study drug at any time during the study should undergo a dose taper (see Section 9.3), unless an emergency situation justifies discontinuing the study drug 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

A minimum of 20, with up to 40, 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
- Plans to modify, suspend, or discontinue the development of the study drug
- 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 study central genotyping laboratory.
4. Have a history of a minimum of 6 transfusion episodes in the 52-week period prior to date of informed consent as documented in the transfusion history of the subject, which reflects the subject's typical transfusion burden.
5. Have complete records of transfusion history, defined as having the following available for the 52 weeks prior to the date of informed consent: (1) **all** the transfusion dates, (2) the number of blood units transfused for **all** the transfusions, and (3) Hb concentrations within 1 week prior to transfusion for at least 80% of the transfusions.
6. Have received at least 0.8 mg oral folic acid daily for at least 21 days prior to the first dose of study drug, 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.
    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.
    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 follicle-stimulating hormone (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 drug for women and 90 days following the last dose of study drug 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 drug.
10. Be willing to comply with all study procedures, in particular the Individual TT (calculated based on 52 weeks of transfusion history), 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 study 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 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. 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 RBC disorders).
i. Positive test for HBsAg 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 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 drug.
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 history of transfusions occurring on average more frequently than once every 3 weeks during the 52 weeks prior to signing informed consent.
4. Have a splenectomy scheduled during the study drug period or have undergone splenectomy within 12 months prior to signing informed consent.
5. 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.
6. Have exposure to any investigational drug, device, or procedure within 3 months prior to the first dose of study drug.
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 drug.
11. Are currently receiving hematopoietic stimulating agents (eg, erythropoietins [EPOs], 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 drug.
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 drug.

8.3. Subject Withdrawal Criteria

Subjects may withdraw from the study at any time for any reason. Subjects will be withdrawn from study drug 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 9.5.1)
- Investigator decision
- Persistent nonadherence to protocol requirements
- Pregnancy
- Lost to follow-up

Subjects who discontinue treatment will be encouraged to return for subsequent scheduled visits (or at a minimum the End of Study Visit) unless they withdraw 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 drug or the study, the Medical Monitor (or designee) must be informed.

When a subject withdraws from the study drug or withdraws from the study, the primary reason for treatment discontinuation or study discontinuation must be recorded in the appropriate section of the 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 who discontinue the study for reasons other than AEs may be replaced at the Sponsor's discretion.

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 Drug

9.1.1. Study Drug

AG-348 will be supplied as 5, 20, and 50 mg strength 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 drug product will be supplied by the Sponsor.

9.1.2. Study Drug Packaging and Labeling

AG-348 will be supplied in appropriate containers with child-resistant closures and will be labeled appropriately as IMP for this study. Packaging and labeling will be prepared to meet all regulatory requirements.

This is an open-label study; there are no additional requirements regarding the physical aspect of blinding related to packaging and labeling.

9.1.3. Study Drug Storage

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

9.1.4. Study Drug Administration

AG-348 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 drug may be taken with or without food. Subjects will take 1 tablet BID, approximately 12 hours apart (ie, 12 hours±2 hours). Subjects will be instructed to complete a dosing diary in the eDiary dispensed during Screening.

If a subject misses a scheduled dose by 4 hours or less, he or she should still take that dose. If a subject misses a scheduled dose by more than 4 hours, he or she should skip that dose. If a dose is skipped, the next dose should then be taken approximately 24 hours from the previous dose. If a subject experiences a dose interruption for non-safety reasons, they should be re-started on the prescribed dose as soon as possible.

Subjects should be advised not to abruptly interrupt or discontinue dosing without first speaking with the treating Investigator except in case of medical emergency; abrupt interruption or discontinuation of AG-348 may result in withdrawal hemolysis. If a subject needs to interrupt or permanently discontinue study drug at any time during the study, guidance is provided in Section 9.3 and Section 7.1.5.

9.1.5. Study Drug Accountability

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

The Investigator or delegate will maintain accurate drug accountability records indicating the drug's delivery date to the site, inventory at the site, use by each subject, and return to the Sponsor or 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 drug 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 drug must not be used for any purpose other than the present study.

All unused and used study drug will be retained at the site until it is inventoried by the Site Monitor. All used, unused, or expired study drug 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 drug is expected to be dispensed to the subject at the study site. If necessary, in exceptional circumstances and with agreement of the Sponsor (or representative), study drug can be provided to the subject's home, if acceptable by practice and allowed by local regulations.

9.1.6. Study Drug Handling and Disposal

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

9.2. Treatment Assignment

All subjects enrolled in this study will receive AG-348.

9.3. Criteria for Dose Reduction and Interruption of Study Drug for Safety Reasons

It is important that a subject does not abruptly interrupt study drug due to the risk of withdrawal hemolysis, except in case of medical emergency.

The Investigator will monitor all subjects for safety. A subject's dose may be reduced (to 5 mg BID or 20 mg BID) or interrupted for safety reasons (ie, excessive Hb response or AEs considered related to study drug) as described below. Guidelines for dose modification for AEs considered related to study drug (except for events of excessive Hb response) are provided in Table 1. After careful consideration of the relative risk of maintaining the subject on the study drug versus withdrawal hemolysis when stopping study drug abruptly versus reducing the dose rapidly, the Investigator should determine if a gradual dose taper (GDT; Table 3) usually indicated for the management of nonacute AEs, a rapid dose taper (RDT; Table 2) usually indicated for the management of acute AEs or SAEs, or abrupt discontinuation of study drug usually indicated to receive emergency medical treatment is necessary. In all cases, study drug dose reductions, interruptions, or discontinuations should be discussed with the Medical Monitor (or designee).

For a subject with an excessive Hb response higher than 2 g/dL (1.24 mmol/L) below the ULN (ie, higher than 15.0 g/dL [9.31 mmol/L] in men and 13.5 g/dL [8.38 mmol/L] in women) up to the ULN (ie, 17.0 g/dL [10.55 mmol/L] in men and 15.5 g/dL [9.62 mmol/L] in women), a dose decrease should be considered for the subject to the next lower dose level, without a need for taper (ie, if the subject experienced 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 taper (ie, if the subject experienced 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.

Table 1: Dose Modification for Adverse Events Considered Related to Study Drug (Except for Events of Excessive Hemoglobin Response), Study AG348-C-007

Related Adverse Event(s) Severity	Dose Modification
Grade 1	None required.
Grade 2	None required. Contact the Medical Monitor (or designee) to discuss specific cases that may need to be managed as Grade 3 events (see below).
Grade 3	After careful consideration of the relative risk of maintaining the subject on the study drug versus the risk of withdrawal hemolysis when stopping AG-348 abruptly or reducing the dose rapidly, the Investigator should determine which of the following options is appropriate: • Maintaining the current dose, or • Performing a dose taper, or • Stopping the study drug abruptly If the decision is made to maintain the current dose of study drug, 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, stopping the study drug abruptly or performing a dose taper should be considered. If the decision is made to stop the study drug abruptly or perform a dose taper, the below instructions should be followed for re-introduction or escalation of the study drug, respectively. **In all cases, re-introduction and escalation of study drug should be performed only after discussion with the Medical Monitor (or designee).[1]** • Restarting treatment after dosing was stopped: Once the event resolves to baseline or Grade 1 (whichever is lower) and the decision is made to restart AG-348, the study drug should be re-introduced at the 5 mg BID dose level. If the event does not reoccur 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 reoccur 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. • Events resolving during the dose taper (ie, the subject is still on study

Related Adverse Event(s) Severity	Dose Modification
	drug): If during the dose taper, the event resolves to baseline or Grade 1 (whichever is lower), the study drug should be maintained at the dose at which the event resolved for at least 4 weeks (with at least once weekly monitoring). If the event does not reoccur after at least 4 weeks, then it should be considered to increase the dose 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 reoccur after at least 4 weeks (with at least once weekly monitoring), and the subject is not already receiving 50 mg BID, then it should be considered to increase the dose to the next BID dose level. If the AE reoccurs at any point during the above scenarios, the subject should undergo a dose taper or stop AG-348 abruptly if necessitated by the risk of the AE. If the subject undergoes a dose taper and the AE resolves during the taper, study drug 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 Medical Monitor (or designee). If the AE does not resolve to baseline or Grade 1 (whichever is lower) after the dose is decreased, it should be considered to decrease the dose further. If the AE still does not resolve, dose should be permanently discontinued.
Grade 4	After careful consideration of the relative risk of withdrawal hemolysis when stopping AG-348 abruptly versus reducing the dose rapidly, the Investigator should determine which of the following options is appropriate: • Performing a rapid dose taper **or** • Stopping the study drug abruptly If the event resolves and the Investigator believes that restarting study drug is justified, the Medical Monitor (or designee) should be consulted before any further study drug is administered.

Abbreviations: AE = adverse event; BID = twice daily; Hb = hemoglobin; RDT = rapid dose taper.
[1] The study drug dose should not be increased if Hb concentration (per the central laboratory analysis) is ≥14.5 g/dL (9.0 mmol/L) in males or ≥13.0 g/dL (8.07 mmol/L) in women.

9.3.1. Rapid Dose Taper

In subjects undergoing an RDT, the regimen as detailed in Table 2 will be followed. Subjects going through the RDT should be monitored for signs of hemolysis and worsening of anemia.

Table 2: Rapid Dose Taper Regimen, Study AG348-C-007

Starting Dose	First Step ×3 days	Second Step ×3 days	Third Step ×3 days
5 mg BID	5 mg QD	5 mg QOD	n/a
20 mg BID	20 mg QD	20 mg QOD	n/a
50 mg BID	50 mg QD	20 mg QD	20 mg QOD

Abbreviations: BID = twice daily; n/a = not applicable; QD = once daily; QOD = every other day.

9.3.2. Gradual Dose Taper

In subjects undergoing a GDT, the regimen detailed below and in Table 3 will be followed.

For subjects on either 5 mg BID or 20 mg BID of study drug, the GDT will start with subjects reducing the frequency of dosing from their usual regimen of 1 tablet BID to 1 tablet QD for 7 days, followed by 1 tablet QOD for 7 days prior to stopping study drug altogether (see Table 3).

For subjects on 50 mg BID, the GDT will start with subjects reducing the frequency of dosing from their usual regimen of 1 tablet BID to 1 tablet QD for 7 days; then they will switch to 1 tablet of 20 mg QD for 7 days, followed by 1 tablet 20 mg QOD for 7 days prior to stopping study drug altogether (see Table 3). Dose tapers may be discussed with the Medical Monitor (or designee).

Subjects going through a GDT should be monitored for signs of hemolysis and worsening of anemia. If a GDT is performed to discontinue dosing permanently, subjects may stop taking the study drug after the taper has been completed.

Table 3: Gradual Dose Taper Regimen, Study AG348-C-007

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

Abbreviations: BID = twice daily; n/a = not applicable; QD = once daily; QOD = every other day.

9.4. Transfusions

Transfusion history should be recorded as noted in Section 7.1.2.1.

Transfusion(s) during the Screening Period will be administered according to the usual care of the subject (ie, without regard to the subject's Individual TT) and will be recorded but not included in the subject's transfusion history. During the Screening Period, the last transfusion should be administered 2 to 7 days before Day 1.

For each transfusion administered at any time during the study, date of transfusion, number of RBC units transfused, HCT, and volume of RBC units will be recorded.

9.5. Prior and Concomitant Medications

Prior medications are defined as those administered anytime with the 28 days prior to signing of the ICF until the first dose of study drug. Concomitant medications are defined as those administered from the point of first dose of study drug 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 9.6.

9.5.1. 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 drug.

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

Also, AG-348 exhibits pH-dependent solubility. Therefore, proton-pump inhibitors and H_2-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 Appendix 5 of the AG-348 IB)
- Products known to inhibit CYP3A4, such as grapefruit or grapefruit juice
- Strong inducers of CYP3A4 (listed in Appendix 5 of the AG-348 IB)
- Strong inhibitors of P-gp (listed in Appendix 5 of 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, EPOs, granulocyte colony stimulating factors, thrombopoietins) must be discontinued no less than 28 days prior to the first dose of study drug. 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 drug.

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 subjects 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 Appendix 5 of the AG-348 IB)
- Moderate inhibitors of CYP3A4 (listed in Appendix 5 of the AG-348 IB)
- Sensitive substrates of CYP2B6 (listed in Appendix 5 of the AG-348 IB)
- Proton-pump inhibitors and H_2-receptor antagonists (listed in Appendix 5 of 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 contraceptive while enrolled in the study and until at least 28 days after their last dose of study drug, as specified in Inclusion Criterion #9 (see Section 8.1).

9.5.2. Allowed Concomitant Medications

Medications other than those specified above (Section 9.5.1) 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 9.5.1.

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.

9.6. 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 #4) 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.

9.7. Treatment Compliance

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

9.8. Randomization and Blinding

This is an open-label study, and no blinding is involved. All subjects will receive AG-348 as described in Section 9.2. There is no randomization.

10. STUDY ASSESSMENTS

The timing of all study assessments is indicated in the Schedule of Assessments for Part 1 (Table 4) and Part 2 (Table 5) of the study.

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). A subject will not be considered enrolled (and cannot receive his/her first dose of study drug) until eligibility is confirmed.

If a subject is determined to be ineligible for the study, the subject will be excluded from participation.

If study ineligibility is due to a transient condition (eg, prohibited concomitant medication), the subject could be rescreened after the criterion that the subject failed to meet has resolved. In the case of a subject declared ineligible according to a previous version of the protocol, the subject 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 Screening: transfusion history unless additional transfusions have been administered, demographics, medical/surgical history unless new information needs to be added, liver MRI unless done more than 6 months prior, DXA scan unless done more than 6 months prior, and *PKLR* genotyping.

10.3. Medical and 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.

10.3.1. 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.

10.4. Prior and Concomitant Medications

Prior and concomitant medications will be captured as described in Section 9.5 and per the Schedule of Assessments (Section 10.14.1).

10.5. *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.

10.6. Demographics

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

Race and ethnicity data 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.

10.7. Assessment of Efficacy

10.7.1. Transfusion Burden

Transfusion burden (number of RBC units received over a 24-week period) will be assessed based on the transfusion information collected in Part 2 of the study. Information on transfusions administered during the study will be recorded as specified in Section 9.4.

10.8. Assessments of Safety

10.8.1. Vital Signs

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

10.8.2. Physical Examination

Complete physical examinations will be performed according to the Schedules of Assessments (Section 10.14.1) and additionally when clinically indicated, at the discretion of the Investigator.

Height will be documented during Screening only.

10.8.3. Laboratory Assessments

Safety laboratory assessments are described in Section 10.9.1.

10.8.4. Electrocardiogram

The 12-lead ECGs should be performed following 5 minutes of recumbency and in triplicate using the ECG machine provided by the central vendor and according to the vendor manual. The ECG at Part 2 Week 24 will be performed prior to, but within the same window as, the

pharmacokinetic sample collected 1 hour (±5 minutes) after the study drug dose administration to align with the approximate T_{max} (time to maximum [peak] concentration) of the study drug.

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.

10.8.5. 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.

10.8.6. 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.

10.9. Laboratory Assessments

All clinical laboratory evaluations are to be performed by a central laboratory. If Investigators believe that it is clinically indicated to obtain urgent safety laboratory results from their own local laboratories on the day of the subject's visit and before the results are returned from the designated central laboratory, they are free to exercise their discretion to do so. In this case, a sample should be sent, in parallel, to the central laboratory. Decisions about subject eligibility and subjects' management are to be based on the results obtained from the central laboratory, unless an urgent safety issue requires performing a local test, and except for pregnancy testing from Day 1 until the end of the study, which should be done locally.

Blood samples for clinical evaluations will be collected according to the Schedules of Assessments (Section 10.14.1). All blood sample draws for screening tests should be performed as long after a previous transfusion as possible. In addition, all clinically significant laboratory abnormalities noted on testing will be followed by repeat testing and further investigated according to the judgment of the Investigator.

Safety laboratory assessments and other laboratory assessments are listed in Section 10.9.1 and Section 10.9.2, respectively.

10.9.1. Safety Laboratory Assessments

The following safety laboratory parameters will be measured:

Hematology:	Complete blood count with differential (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, red cell distribution width, nucleated RBC count, white blood cell count, ANC, absolute lymphocyte count, absolute monocyte count, eosinophil count, basophil 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, creatinine, and uric acid. At Screening, estimated GFR, measured GFR, or calculated CrCL (Cockcroft-Gault) will be assessed.
Lactate Dehydrogenase and Haptoglobin:	LDH and haptoglobin
Liver Function Tests:	Alkaline phosphatase (ALP), ALT, AST, total bilirubin, direct bilirubin, and indirect bilirubin
Sex Steroid Testing:	Testosterone (total and free), estrone, and estradiol
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 (Human Chorionic Gonadotropin [hCG]) Test:	Serum or urine pregnancy tests (with the exception of the screening pregnancy test, which must be serum)
***UGT1A1* Genotyping:**	To detect subjects with Gilbert's syndrome

For hematology parameters, the most recent nonmissing measurements prior to Transfusion 0 (the most recent transfusion occurring in the Screening Period prior to the start of study drug on Day 1) will be defined as the baseline. This is to limit the impact of transfusion on these hematology laboratory parameters. For transaminase (AST and ALT), the baseline will be defined as the average of the Screening and Day 1 values.

10.9.2. 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 postmenopausal 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: EPO, erythroferrone, soluble transferrin receptor, and other makers of erythropoiesis
- Assessments of complications of iron overload: thyroxine, thyroid-stimulating hormone, parathormone, fructosamine, and vitamin D

All blood sample draws for screening tests should be performed as long after a previous transfusion as possible.

10.10. 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 immediately to a central vendor for technical assessment and analysis. For further details, please refer to the vendor's manual.

10.11. Pharmacokinetic Assessments

10.11.1. Blood Sample Collection

On days of pharmacokinetic blood sample collection, the morning dose of study drug must be administered at the study site. On days where a predose sample is required, the study drug 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 drug):

- Week 24 Visit in Part 2 (full profile blood sampling)
  - Predose (within 1 hour prior to study drug administration)
  - 0.5 hours (±5 minutes) post study drug administration
  - 1 hour (±5 minutes) post study drug administration
  - 2 hours (±5 minutes) post study drug administration
  - 4 hours (±30 minutes) post study drug administration
  - 8 hours (±30 minutes) post study drug 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.

10.11.2. 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 measurable 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

10.12. Pharmacodynamic Assessments

Pharmacodynamic samples to measure PKR protein levels in whole blood will be taken during Screening between Day -7 and Day -2 before Transfusion 0 is administered, at the Day 1 Visit in Part 1 before administration of the first dose, and during Part 2 at the Week 24 Visit. 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.

On days of sample collection (except Transfusion 0), the morning dose of study drug must be administered at the study site following the predose collections. The predose sample for PD assessments during Part 2 at the Week 24 Visit should be collected within 60 minutes prior to study drug administration. Procedures for sample collection and processing will be provided in a separate study manual.

10.13. Health-Related Quality of Life Assessments

Subjects will use the eDiary (provided during Screening) to record responses to each of the HRQoL assessments (Section 10.13.1, Section 10.13.2, and Section 10.13.3). 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 10.14.1).

10.13.1. Pyruvate Kinase Deficiency-Specific Health-Related Quality of Life 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.

10.13.2. EuroQol-5D-5L

The EQ-5D-5L is a standardized instrument for evaluating quality of life 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.

10.13.3. Patient Global Impression of Severity

The Patient Global Impression of Severity (PGIS) is a single-item questionnaire used to rate the subject's impression of the severity of his/her condition.

10.14. Study Conduct

10.14.1. Schedule of Assessments

The Schedule of Assessments for Part 1 of the study is in Table 4 and the Schedule of Assessments for Part 2 of the study is in Table 5.

Table 4: Schedule of Assessments – Screening and Part 1 of Study AG348-C-007

Visit:	**Screening**[1]		**P1D1**[2]	**P1W2**	**P1W4**	**P1W6**	**P1W8**	**P1W10**	**P1W12**	**P1W14**	**P1W16**[3]
Study Day: (Window [days])	**D -56 to D -7**	**Transfusion 0**[4] **D -7 to D -2**	**D1**	**D15 (±3 d)**	**D29 (±3 d)**	**D43 (±3 d)**	**D57 (±3 d)**	**D71 (±3 d)**	**D85 (±3 d)**	**D99 (±3 d)**	**D113 (±3 d) (P1W16 is the same day as P2D1)**[3]
Informed consent	X										
Transfusion history[5]	X										
Demographics	X										
Medical/surgical history[6]	X										
Prior medications	X										
Physical examination[7]	X		X						X		X
Vital signs[8]	X		X	X	X	X	X	X	X	X	X
12-lead ECG[9]	X		X								X
Liver MRI to assess LIC[10]	X										X
DXA scan[10]	X										X
Dispense eDiary[11]	X										
HRQoL assessments[11,12]	X		X		X		X		X		X
Dosing diary[11]			X								
Menstrual cycle diary[11]	X										
PKLR genotyping[13]	X										
Clinical laboratory evaluations[14]											
HBsAg, HCVAb, HIV-1, and -2 Ab[15]	X										
FSH[16]	X										

Visit:	**Screening**[1]		**P1D1**[2]	**P1W2**	**P1W4**	**P1W6**	**P1W8**	**P1W10**	**P1W12**	**P1W14**	**P1W16**[3]
Study Day: (Window [days])	**D -56 to D -7**	**Transfusion 0**[4] **D -7 to D -2**	**D1**	**D15 (±3 d)**	**D29 (±3 d)**	**D43 (±3 d)**	**D57 (±3 d)**	**D71 (±3 d)**	**D85 (±3 d)**	**D99 (±3 d)**	**D113 (±3 d) (P1W16 is the same day as P2D1)**[3]
Hematology[17]	X	X	X	X	X	X	X	X	X	X	X
Serum chemistry[18]	X		X						X		X
Liver function tests[19]	X		X	X	X	X	X	X	X	X	X
LDH and haptoglobin	X		X	X	X	X	X	X	X	X	X
T4, TSH, PTH, fructosamine, and vitamin D			X								
Coagulation studies[20]	X		X						X		
Urinalysis[21]	X		X						X		
Pregnancy test[22]	X		X		X		X		X		X
Iron panel and related markers[23]	X		X						X		
Markers of erythropoietic activity[23]	X		X						X		
Lipids[24]			X			X			X		X
Sex steroids[25]			X		X		X		X		X
PKR protein[26]		X	X								
Eligibility confirmation[27]			X								
UGT1A1 genotyping[28]			X								
Dispense study drug[29]			X	X	X	X	X	X	X	X	X
Study drug dosing			X								
Return study drug				X	X	X	X	X	X	X	X

Visit:	**Screening**[1]		**P1D1**[2]	**P1W2**	**P1W4**	**P1W6**	**P1W8**	**P1W10**	**P1W12**	**P1W14**	**P1W16**[3]
Study Day: (Window [days])	**D -56 to D -7**	**Transfusion 0**[4] **D -7 to D -2**	**D1**	**D15 (±3 d)**	**D29 (±3 d)**	**D43 (±3 d)**	**D57 (±3 d)**	**D71 (±3 d)**	**D85 (±3 d)**	**D99 (±3 d)**	**D113 (±3 d) (P1W16 is the same day as P2D1)**[3]
Concomitant medications/ transfusions[30]			X								
SAEs/AEs/AESIs[31]	X										

Abbreviations: Ab = antibody; AE = adverse event; AESI = adverse event of special interest; ALC = absolute lymphocyte 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; CO_2 = carbon dioxide; CrCL = calculated creatinine clearance; CRP = C-reactive protein; D = Study Day; d = days; DXA = dual-energy X-ray absorptiometry; ECG = electrocardiogram; EQ-5D-5L = EuroQol-5D-5L; FSH = follicle-stimulating hormone; GFR = glomerular filtration rate; Hb = hemoglobin; HBsAg = hepatitis B surface antigen; hCG = human chorionic gonadotropin; HCV = hepatitis C virus; HCT = hematocrit; HDL-C = high-protein 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; 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 cell count; NTBI = non-transferrin bound iron; P = Part; 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 = parathormone; RBC = red blood cell; RDW = red cell distribution width; RT-PCR = reverse transcriptase-polymerase chain reaction; SAE = serious adverse event; TIBC = total iron-binding capacity; TSH = thyroid-stimulating hormone; T4 = thyroxine; *UGT1A1* = uridine diphosphate glucuronosyltransferase 1A1 gene; W = Week; WBC = white blood cell.

Note: Whenever more than 1 assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (eg, vital signs, ECG, blood draw). The timing of these assessments should allow the blood draw to occur at the exact nominal time (if applicable)

[1] A subject's Screening Period duration may be extended beyond 8 weeks upon the Medical Monitor's (or designee's) approval if there is a delay in obtaining a subject's complete transfusion history or the subject is projected to need a transfusion within 1 week of his/her scheduled first dose to ensure that treatment with AG-348 is always started 2-7 days after a subject's most recent transfusion in the Screening Period (referred to as Transfusion 0). All blood sample draws for screening tests should be performed as long after a previous transfusion as possible.

[2] The Day 1 visit should take place 2-7 days after the last transfusion in the Screening Period (ie, Transfusion 0). The first dose of study drug 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 Part 1 Week 16 Visit will mark the transition from Part 1 into Part 2 (ie, Part 1 Week 16 is the same as Part 2 Day 1, marking the end of Part 1 and the beginning of Part 2).

[4] Some subjects may require more than 1 transfusion during the Screening Period. Transfusion 0 is defined as the most recent transfusion occurring in the Screening Period prior to the start of study drug on Day 1. This transfusion does not need to be conducted at the study site, however blood samples indicated in the table will be collected and sent to the central laboratory for analysis. These samples must be drawn before Transfusion 0 is administered and within the Day -7 to Day -2 window.

[5] The complete transfusion history (as defined in Section 7.1.2.1) for the entire 52-week period prior to informed consent must be collected and will be used to inform study eligibility and analyze data.

[6] All medical and surgical history deemed to be relevant per the Investigator (in particular, pertaining, but not limited to, the diagnosis of PK deficiency), current medical conditions, and prior history of splenectomy and/or cholecystectomy are to be recorded. 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.

[7] A complete physical examination will be performed. Height will be documented during Screening only. Additional physical examinations may be performed when clinically indicated, at the discretion of the Investigator.
[8] Vital signs of systolic and diastolic BP, heart rate, and body temperature will be collected.
[9] The 12-lead ECGs will be conducted using the equipment provided by the vendor and according to the vendor manual after 5 minutes of recumbency 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.
[10] The DXA and liver MRI scan(s) 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 drug is administered.
[11] 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 change in characteristics) in the eDiary; the menstrual diary can be completed at any time of day. The dosing diary should be completed every day in the evening (between 5:00-11:00 PM).
[12] Health-related quality of life will be assessed using PKDD, PKDIA, EQ-5D-5L, and PGIS. Note that PGIS is collected at Day 1 only. The HRQoL assessments should be completed in the evening (between 5:00-11:00 PM) within the window for the relevant study visit.
[13] For eligibility confirmation, the *PKLR* genotyping must be analyzed by the central laboratory; sample must be drawn early in the Screening Period to ensure results are back in time to allow the Investigator to assess eligibility.
[14] On Day 1 of Part 1, all clinical laboratory evaluations should be collected predose. All clinical laboratory evaluations should be analyzed centrally, except the urine or serum pregnancy tests performed after Screening. For an urgent safety issue that may require local laboratory evaluations, refer to Section 10.9 of the protocol.
[15] 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.
[16] 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 the ICF). Samples should be drawn in the morning (does not need to be fasting).
[17] Hematology parameters (ie, CBC with differential) will 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, absolute monocyte count, eosinophil count, basophil count, and platelet count.
[18] Serum chemistry parameters will be collected after an overnight fast (with the exception of the screening assessment, which can be done without fasting) and will 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 CrCL (Cockcroft-Gault) will be assessed. If the subject reports that he/she did not adhere to an overnight fast, these samples should not be collected at the scheduled visit (with the exception of the screening assessment, which can be done without fasting). 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.
[19] Liver function tests are ALP; ALT; AST; and total, direct, and indirect bilirubin.
[20] Coagulation parameters will include fibrinogen, aPTT, and INR.
[21] Urinalysis will be performed by a dipstick method and will include assessments of protein, glucose, leukocytes, and blood.
[22] A serum pregnancy (hCG) test must be done at Screening and sent to the central laboratory. A urine or serum pregnancy (hCG) test must be done and documented to be negative on Day 1 before administration of the first dose of AG-348. A urine or serum pregnancy (hCG) test must be repeated every 4 weeks at the indicated visits and must also be done 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.
[23] The iron panel and related markers will 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).
[24] For lipid testing samples for total cholesterol, HDL-C, LDL-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.
[25] Sex steroid testing will assess testosterone (total and free), estrone, and estradiol. Samples should be drawn in the morning (does not need to be fasting).

[26] Samples for PKR protein are to be collected prior to Transfusion 0 and predose on Day 1. On days of sample collection (except for Transfusion 0), the morning dose of study drug must be administered at the study site following the predose collections.
[27] 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 the Screening Period. In addition, on Day 1, the site must verify that a subject who has been considered eligible after completing Screening has a negative urine or serum pregnancy test, if applicable, before the subject can be dosed.
[28] *UGT1A1* genotyping results are not required prior to enrollment.
[29] Study drug is expected to be dispensed to the subject at the study site. If necessary, in exceptional circumstances and with agreement of the Sponsor (or representative), study drug can be provided to the subject's home, if acceptable by practice and allowed by local regulations.
[30] Collection of transfusion information for transfusions administered during the study will include the number of transfusions, date of the transfusions, HCT, volume of RBC units, and the number of RBC units transfused.
[31] The Investigator should ask the subject for information regarding sleep patterns, signs, and symptoms associated with insomnia.

Table 5: Schedule of Assessments – Part 2 of Study AG348-C-007

Visit:	**P2W4**	**P2W8**	**P2W12**	**P2W18**	**P2W24 or EOS[1]**	**Follow-up[2]**
Study Day: (Window [days])	**D29 (±3 d)**	**D57 (±3 d)**	**D85 (±3 d)**	**D127 (±3 d)**	**D169[1] (±3 d)**	**28 d after the last dose[2] (±4 d)**
Physical examination[3]			X		X	
Vital signs[4]	X	X	X	X	X	X
12-lead ECG[5]					X	X
Liver MRI to assess LIC[6]					X	
DXA scan[6]					X[6]	
HRQoL assessments[7,8]	X	X	X	X	X	X[2]
Dosing diary[8]	X					
Menstrual diary[8]	X					
Clinical laboratory evaluations[9]						
Hematology[10]	X	X	X	X	X	X
Serum chemistry[11]			X		X	X
Liver function tests[12]	X	X	X	X	X	X
LDH and haptoglobin	X	X	X	X	X	X
T4, TSH, PTH, fructosamine, and vitamin D					X	
Coagulation studies[13]			X		X	
Urinalysis[14]			X		X	X
Iron panel and related markers[15]			X		X	
Markers of erythropoietic activity[15]			X		X	
Lipids[16]	X	X	X	X	X	X
Sex steroids[17]	X	X	X	X	X	X
Pregnancy tests[18]	X	X	X	X	X	X

Visit:	**P2W4**	**P2W8**	**P2W12**	**P2W18**	**P2W24 or EOS[1]**	**Follow-up[2]**
Study Day: (Window [days])	**D29 (±3 d)**	**D57 (±3 d)**	**D85 (±3 d)**	**D127 (±3 d)**	**D169[1] (±3 d)**	**28 d after the last dose[2] (±4 d)**
Blood sampling for pharmacokinetic profile[19]					X[1]	
PKR protein[20]					X[1]	
Dispense study drug[21]	X	X	X	X	X[1]	
Study drug dosing	X					
Return study drug	X	X	X	X	X[1]	X
Return eDiary					X[1]	X
Concomitant medications/transfusions[22]	X					
SAEs/AEs/AESIs[23]	X					

Abbreviations: AE = adverse event; AESI = adverse event of special interest; ALC = absolute lymphocyte 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; CO_2 = carbon dioxide; CRP = C-reactive protein; D = Study Day; d = days; DXA = dual-energy X-ray absorptiometry; ECG = electrocardiogram; EOS = end of study; EQ-5D-5L = EuroQol-5D-5L; Hb = hemoglobin; hCG = human chorionic gonadotropin; HCT = hematocrit; HDL-C = high-density lipoprotein cholesterol; HRQoL = health-related quality of life; ICF = Informed Consent Form; INR = international normalized ratio; LDH = lactate dehydrogenase; LDL-C = low-density lipoprotein cholesterol; 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 cell count; NTBI = non-transferrin bound iron; P = Part; 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 = parathormone; RBC = red blood cell; RDW = red cell distribution width; SAE = serious adverse event; T4 = thyroxine; TIBC = total iron-binding capacity; T_{max} = Time to maximum (peak) concentration; TSH = thyroid-stimulating hormone; W = Week; WBC = white blood cell.

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

[1] Subjects who continue study drug through Part 2 Week 24 should continue taking study drug at least through the morning dose of the Part 2 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 with AG-348. Subjects who discontinue the study prior to the Part 2 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 drug, whichever is later. Note, this may occur at any time during the study (Part 1 and Part 2). The End of Study Visit will be identical to the Part 2 Week 24 Visit with these exceptions: no study drug will be dispensed, blood sampling for pharmacokinetic profile will not be drawn, PKR protein will not be assessed, and if the subject has not already done so, they should return their eDiary and study drug.

[2] Subjects who continue study drug through the Part 2 Week 24 Visit but do not continue on into an extension study should undergo a dose taper and then attend the Follow-up Visit 28 ±4 days after the last dose of study drug (with the exception of HRQoL assessments, which will occur 28±4 days after the subject's last full dose of study drug). Subjects who continue on into an extension study will not be required to attend the Follow-up Visit. Subjects who initiate or are undergoing a dose taper for a reason related to safety and for whom participation in an extension study remains undetermined should perform the taper, and a transition to an extension study should be discussed with the Medical Monitor (or designee).

[3] A complete physical examination will be performed. Additional physical examinations may be performed when clinically indicated, at the discretion of the Investigator.
[4] Vital signs of systolic and diastolic BP, heart rate, and body temperature will be collected.
[5] The 12-lead ECGs will be conducted using the equipment provided by the vendor and according to the vendor manual after 5 minutes of recumbency 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 Part 2 Week 24 will be performed prior to, but within the same window as, the pharmacokinetic sample collected 1 hour (±5 minutes) after the study drug dose administration to align with the approximate T_{max} of the study drug. The ECG performed as part of the EOS Visit can be collected at any time during the visit.
[6] The DXA and liver MRI scan(s) should be performed per the vendor manuals. If the DXA and/or MRI scan(s) are deemed to be of low quality by the central vendor, the scan(s) will be repeated no later than the next visit. The DXA scan for the EOS Visit will be performed only for these subjects if they have been on AG-348 for at least 22 weeks (since Part 1 Day 1).
[7] Health-related quality of life will be assessed using PKDD, PKDIA, EQ-5D-5L, and PGIS. Note that PGIS should be collected only at the Week 24 Visit or the EOS Visit (if applicable). The HRQoL assessments should be completed in the evening (between 5:00-11:00 PM) within the window for the relevant study visit.
[8] All subjects will record responses to HRQoL assessments and keep a dosing diary in an eDiary dispensed at Screening. In addition, menstruating female subjects will record their menstrual cycles (start date, stop date, and characteristics) in the eDiary; the menstrual diary can be completed at any time of day. The dosing diary should be completed every day in the evening (between 5:00-11:00 PM).
[9] All clinical laboratory evaluations should be analyzed centrally, except the urine or serum pregnancy tests. For an urgent safety issue that may require local laboratory evaluations, refer to Section 10.9 of the protocol.
[10] Hematology parameters (ie, CBC with differential) will 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, absolute monocyte count, eosinophil count, basophil count, and platelet count.
[11] Serum chemistry parameters will be collected after an overnight fast and will include sodium, potassium, chloride, calcium, magnesium, phosphorus, CO_2 or bicarbonate, albumin, total protein, glucose, BUN or urea, creatinine, and uric acid. If the subject reports that he/she 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.
[12] Liver function tests are ALP; ALT; AST; and total, direct, and indirect bilirubin.
[13] Coagulation parameters will include fibrinogen, aPTT, and INR.
[14] Urinalysis will be performed by a dipstick method and will include assessments of protein, glucose, leukocytes, and blood.
[15] The iron panel and related markers will 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).
[16] For lipid testing samples for total cholesterol, HDL-C, LDL-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.
[17] Sex steroid testing will assess testosterone (total and free), estrone, and estradiol. Samples should be drawn in the morning (does not need to be fasting).
[18] A urine or serum pregnancy (hCG) test must be repeated every 4-6 weeks at the indicated visits and must also be done 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.
[19] Plasma samples for pharmacokinetic analysis will be collected at the following time points: predose (within 1 hour prior to dosing) and 0.5 hour (±5 minutes), 1 hour (± 5 minutes), 2 hours (±5 minutes), 4 hours (±30 minutes), and 8 hours (±30 minutes) postdose. On days of pharmacokinetic blood sample collection, the morning dose of study drug must be administered at the study site; on days where a predose sample is required, the study drug must be administered after the predose sample is taken.
[20] Samples for PKR protein are to be collected predose (within 60 minutes prior to study drug administration). On days of sample collection, the morning dose of study drug must be administered at the study site following the predose collections.

[21] Study drug is expected to be dispensed to the subject at the study site. If necessary, in exceptional circumstances and with agreement of the Sponsor (or representative), study drug can be provided to the subject's home, if acceptable by practice and allowed by local regulations.
[22] Collection of transfusion information for transfusions administered during the study will include the number of transfusions, date of the transfusions, HCT, volume of RBC units, and the number of RBC units transfused.
[23] The Investigator should ask the subject for information regarding sleep patterns, signs, and symptoms associated with insomnia.

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 drug unless the subject initiates 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 initiates 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 drug 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 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 NCI 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 drug. 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 drug-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 the 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 Adverse Event 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:

a. Accompanied by clinical symptoms
b. Results in a change in study drug (eg, dosage modification, treatment interruption, or treatment discontinuation)
c. Results in a medical intervention (eg, potassium supplementation for hypokalemia) or a change in concomitant therapy
d. 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 average 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) Abs, cytomegalovirus (CMV) Abs, Hepatitis A Ab, HBsAg, HCVAb (with an RT-PCR test performed if HCVAb is positive), HIV-1Ab, and HIV-2Ab using the central laboratory.
3. Autoimmune hepatitis panel consisting of the following: serum antinuclear antibody, antismooth muscle antibody, liver-kidney microsomal type 1 antibody, antibody to

soluble liver antigen, and antimitochondrial antibody when transaminase increase meets the criteria of AESI and repeated 4 weeks later using the central laboratory if the results were negative in the first time.

The Investigator should refer to Section 9.3 to determine if a dose adjustment is needed. If the Investigator is not sure whether or not a dose adjustment is needed, they should consult with the Medical Monitor (or designee).

11.3. 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, physical examination, 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 drug.

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 gender-specific ULN). 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 is 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 Drug

Relationship to study drug 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 drug (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 drug (eg, cancer diagnosed 2 days after the first dose of study drug).
- 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 drug, 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 drug; and/or
  - The AE abates or resolves upon discontinuation of the study drug 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 AG-348 must be reported to the Sponsor or 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 AG-348, must be reported to the Sponsor or 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 Sponsor 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 drug for women and 90 days following the last dose of study drug for men. Periodic abstinence (eg, calendar, symptothermal, and post-ovulation methods) and withdrawal are not acceptable methods of contraception.

12. STATISTICAL METHODS

12.1. Sample Size and Power

Due to the rarity of the disease and the small patient population, the sample size is largely driven by feasibility. Given the feasibility results and uncertainty of the screen failure rate, the study will enroll a minimum of 20, with up to 40, subjects. With a sample size of 20, the power of the study is limited, that is, the power will be 58% to detect a response rate of 30% compared to a null rate of 10% with an exact test at 2-sided 0.05 significance level. There will be 75% power to detect a large response rate of 35%, compared to the null of 10%. When the sample size is 40, the power will be at least 90%, even if the target response rate is 30%. The power for different scenarios is listed in Table 6.

Table 6: Power Calculation

Sample Size	Target Response Rate (null=10%)		
	25%	30%	35%
20	0.38	0.58	0.75
30	0.48	0.71	0.87
40	0.71	0.90	0.97

The probability of observing at least 1 AE based on different true incidence rate and sample sizes is displayed in Table 7.

Table 7: Probability of Observing ≥1 Adverse Event Based on True Incidence Rate and Sample Size

Sample Size	AE Incidence	
	5%	10%
20	64.2%	87.8%
30	78.5%	95.8%
40	87.1%	98.5%

Abbreviation: AE = adverse event.

12.2. Analysis Sets

Assignment of subjects to analysis sets will be done before the clinical database lock for the study. All Subjects will refer to all subjects who were enrolled or dosed (ie, all subjects in the study).

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

12.2.1. Full Analysis Set

The FAS is defined as all subjects who received at least 1 dose of study drug.

The FAS will be used for primary efficacy analyses and safety analyses unless specified otherwise. Additional information may be further provided by the optimized dose.

12.2.2. Per Protocol Set

The PPS is defined as a subset of the FAS including subjects who have completed Part 2.

The PPS will be used for sensitivity analysis as specified in Section 12.3.4.1.

12.3. Statistical Analysis

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

12.3.1. 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 (min), and maximum value (max).

Categorical variables will be summarized using counts and percentages.

Baseline value: Unless otherwise specified, the baseline value will be defined as the most recent nonmissing measurement (scheduled or unscheduled) collected before the initial administration of study drug.

- For hematology parameters, the most recent nonmissing measurements prior to Transfusion 0 (the most recent transfusion occurring in the Screening Period prior to the start of study drug on Day 1) will be defined as the baseline. This is to limit the impact of transfusion on these hematology laboratory parameters.
- Historical transfusion frequency will be based on the historical data during the 52 weeks prior to screening. Details are specified in Section 12.3.2.
- For transaminases (AST and ALT), the baseline will be defined as the average of the assessment of the Screening 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 from the first dose of study drug to 28 days after the last dose of study drug.

12.3.2. Background Characteristics

Subject disposition, demographic and baseline characteristics, prior and concomitant medications, and other background characteristics will be summarized. Additionally, all subject

data will be presented in subject data listings. All summaries will be based on the FAS unless otherwise specified in the SAP.

12.3.2.1. Subject Disposition

Number and percentage of subjects in the following categories will be summarized as appropriate:

- Enrolled
- Dosed (FAS)
- Completed treatment in Part 1
- Prematurely discontinued treatment in Part 1 and the reasons for discontinuation
- Completed treatment in Part 2 (PPS)
- Prematurely discontinued treatment in Part 2 and the reasons for discontinuation
- Completed study
- Prematurely discontinued the study and the reasons for discontinuation

12.3.2.2. Demographics and Baseline Characteristics

Demographic, background (eg, medical history), and baseline characteristics will be summarized. The demographics, baseline characteristics, and medical history summary will be presented for the FAS.

12.3.2.3. Prior and Concomitant Medications

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

- **Prior medication:** any medication that started before initial dosing of study drug, 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, if the subject is still enrolled in the study

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. Post-treatment medications will be listed for each subject.

12.3.3. Study Drug Exposure and Compliance

12.3.3.1. Study Drug Exposure

Duration of study drug 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 drug is missing, the subject's treatment discontinuation or completion date will be used for analysis purposes.

Duration of study drug exposure will be summarized descriptively as a quantitative variable (n, mean, SD, median, min, and max).

Exposure summaries will be based on FAS.

12.3.3.2. Study Drug Compliance

Study drug 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 × total number of single days on study drug / (duration of study drug exposure + total number of days study drug interrupted after last dose, if any)

Single day on study drug is collected from the subject's diary data. If it is unknown whether the subject received treatment on a specified day, then it will be assumed that no treatment was taken on that day.

Percentage of tablets taken will be calculated as follows:

100 × (total number of tablets administered) / (total number of tablets intended)

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

Study drug compliance will be based on the FAS.

12.3.4. Efficacy Analysis

12.3.4.1. Analysis of Transfusion Reduction

Historical transfusion RBC units standardized to 24 weeks will be calculated as the total number of transfusion RBC units over the 52 weeks prior to the informed consent × 24/52. The Part 2 on-study 24-week transfusion RBC units will be calculated as the total number of transfused RBC units received from the first day in Part 2 until the last full dose before starting the dose taper (if applicable) in Part 2 divided by corresponding number of days × 168 (24 weeks).

Both the historical transfusion RBC units standardized to 24 weeks and on-study 24-week transfusion RBC units will be summarized using descriptive statistics (n, mean, SD, median, min, and max). The change in transfusion RBC units (calculated as the on-study 24-week transfusion RBC units – historical transfusion RBC units standardized to 24 weeks) and the percentage change (calculated as [on-study 24-week transfusion RBC units – historical transfusion RBC units standardized to 24 weeks] / historical transfusion RBC units standardized to 24 weeks) will also be summarized based on the FAS. In addition, the number and percentage

of subjects with different categories (<0, ≥0 to <20%, ≥20% to <33%, ≥33% to 50%, ≥50%) of percentage change will also be provided.

Primary Analysis

The number and proportion of subjects who achieve a transfusion reduction response (TRR), defined as ≥33% reduction in the number of RBC units transfused during the 24 weeks in Part 2 compared to the historical transfusion burden standardized to 24 weeks (Standardized Control Period), along with its 95% CI based on the exact binomial distribution, will be provided based on the FAS. Subjects who completed at least 12 weeks of treatment in Part 2 will be evaluated for their transfusion reduction status based on change in transfusion RBC units. Subjects who discontinue the study before completing 12 weeks of treatment in Part 2 will be considered nonresponders. Given the open-label, single-arm nature of the study and limited power of the study, no formal hypothesis test will be conducted. The focus will be given to descriptive statistics, including both point estimate and the CIs.

The similar set of analyses will be repeated based on PPS to further evaluate the impact of early discontinuation on the efficacy results. Details will be provided in the final SAP.

12.3.4.2. Analysis of Other Secondary Efficacy Endpoints

Annualized total number of RBC units transfused during the study compared with the historical transfusion burden

Annualized total number of RBC units transfused during the study (both Part 1 and Part 2) will be calculated as the total number of RBC units transfused during the study divided by the total number of days from the first dose date until the last day on study × 364.

Both the historical transfusion RBC units and on-study annualized transfusion RBC units will be summarized using descriptive statistics (n, mean, SD, median, min, and max). The change in annualized transfusion RBC units and the percentage change will also be summarized based on the FAS. The number and percentage of subjects with different categories of percentage change will also be provided.

Number of transfusion episodes

Number of transfusion episodes during the Standardized Control Period (24 weeks), and number of transfusion episodes during Part 2 (standardized to 24 weeks), their change and percentage change will be summarized using descriptive statistics. Transfusions received over up to 3 consecutive days will be considered as a single transfusion event.

Proportion of subjects transfusion free

Number and percentage of subjects who are transfusion free in the Fixed-Dose Period (Part 2), along with its 95% CI based on the exact binomial distribution, will be provided.

Number of subjects with normal Hb

Number and percentage of subjects who achieve Hb concentrations in the normal range at least once, 8 weeks or more after a transfusion in Part 2, along with its 95% CI based on the exact binomial distribution, will be provided.

Additional sensitivity analysis, supportive analysis (including additional modeling of the secondary endpoints), and analyses for exploratory endpoints and possibly additional endpoints will be described in the SAP.

12.3.5. Safety Analysis

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

- Incidence of treatment-emergent adverse events (TEAEs)
- Clinical laboratory values
- ECGs (standard 12-lead)
- Vital signs
- Physical examination findings
- DXA scans

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

12.3.5.1. Adverse Events

For analysis purposes, AEs will be classified as pretreatment AEs, TEAEs, or post-treatment AEs.

- **Pretreatment AE:** any AE that started before initial dosing of study drug.
- **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 28 days after the last dose of study drug.

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

The AE analysis will be provided for Part 1 and Part 2 separately as well as combining Part 1 and Part 2. Adverse events 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
- TEAEs leading to death

Summaries will be presented by Medical Dictionary for Regulatory Activities 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 the worst/highest relationship level in the relationship summaries. A separate table will summarize all TEAEs when each of them is considered unique, hereafter referred to as an AE count table.

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

12.3.5.2. Clinical Laboratory Assessments

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

For selected laboratory parameters, the number and percentage of subjects with shift changes from baseline (normal/missing, high, and low according to the reference range) to the highest/lowest laboratory evaluation during the treatment-emergent period will be tabulated. The details will be provided in the SAP.

The number and percentage of subjects with transaminase increases of >2.5 × baseline or those that have increased to ≥Grade 2 (AESI of elevated transaminase as defined in Section 11.2.6.1) 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.

12.3.5.3. 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), and heart rate.

The number and percentage of subjects with shift changes from baseline (normal/missing, clinically insignificant, and potentially clinically significant) to the worst ECG evaluation during the TEAE period will be tabulated.

12.3.5.4. Vital Signs

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

12.3.5.5. Physical Examination

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

12.3.5.6. Dual-Energy X-ray Absorptiometry Scans

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

12.4. Interim and Independent Data Monitoring Committee Analyses

12.4.1. Interim Analysis

No interim analysis is planned, but interim analyses may take place at any time during the study if warranted by the ongoing data and/or deemed necessary by the internal Sponsor team.

12.4.2. Independent Data Monitoring Committee Analysis

No independent data monitoring committee is planned for this study.

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 drug 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/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 be conducted only 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 drug accountability records, study reports, and communications will identify the subject by the assigned subject number. The

Investigator will grant monitor(s) and auditor(s) from the Sponsor or 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, email, and fax).

All unused study drug 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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