Official Title: AN OPEN-LABEL, MULTICENTER, EXTENSION STUDY OF AG-348 IN ADULT SUBJECTS WITH PYRUVATE KINASE DEFICIENCY PREVIOUSLY ENROLLED IN AG-348 STUDIES

NCT ID: NCT03853798

Document Date: Study Protocol Version 4.0: 19 July 2022

CLINICAL STUDY PROTOCOL

AG348-C-011

AN OPEN-LABEL, MULTICENTER, EXTENSION STUDY OF AG-348 IN ADULT SUBJECTS WITH PYRUVATE KINASE DEFICIENCY PREVIOUSLY ENROLLED IN AG-348 STUDIES

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EudraCT Number	2018-003459-39
NCT Number	NCT03853798
Document Version (Date)	Original Protocol, Version 1.0 (06 September 2018) Amendment 1, Version 1.1 (29 January 2019) (Denmark) Amendment 1, Version 1.2 (03 May 2019) (Japan) Amendment 2, Version 2.0 (03 October 2019) Amendment 2, Version 2.1 (15 November 2019) (Japan) Amendment 2, Version 2.2 (26 November 2019) (Denmark) Amendment 3, Version 3.0 (26 August 2020) Amendment 3, Version 3.1 (14 October 2020) (Japan) Amendment 3, Version 3.2 (09 December 2020) (Denmark) Amendment 3, Version 3.3 (10 December 2020) (Germany) Amendment 3, Version 3.4 (20 September 2021) (Denmark) Amendment 4, Version 4.0 (19 July 2022)

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AGIOS PROTOCOL APPROVAL

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

Approved by:

PPD

PPD

Associate Medical Director, Clinical Development
Agios Pharmaceuticals, Inc.

Print/Sign/Date (dd mmm yyyy)

PPD

PPD

Senior Director, Biostatistics
Agios Pharmaceuticals, Inc.

Print/Sign/Date (dd mmm yyyy)

INVESTIGATOR'S AGREEMENT

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

This study will not commence without the prior written approval of a properly constituted Institutional Review Board (IRB)/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)

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2. SYNOPSIS

Name of Sponsor/Company:
Agios Pharmaceuticals, Inc.

Name of Investigational Product:
AG-348

Study Title:
An Open-Label, Multicenter, Extension Study of AG-348 in Adult Subjects with Pyruvate Kinase Deficiency Previously Enrolled in AG-348 Studies

Study Center(s):
This multicenter study will be conducted in multiple countries at sites participating in Studies AG348-C-006 and/or AG348-C-007.

Phase of Development:
3

Objectives:

Primary:

- To evaluate the long-term safety and tolerability of AG-348

Secondary:

- To evaluate the long-term efficacy of AG-348
- To evaluate the efficacy of AG-348 in increasing hemoglobin (Hb) concentrations in subjects who previously received placebo in Study AG348-C-006 (*Cohort 1 only*)
- To determine the effect of AG-348 on health-related quality of life (HRQoL) using patient reported outcomes (PROs)
- To evaluate the pharmacokinetics of AG-348 after oral administration (*Cohort 1 only*)
- To evaluate the relationship between AG-348 pharmacokinetics and safety parameters (*Cohort 1 only*)

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Study Endpoints:

The baseline value is defined as the most recent measurement(s) before the first dose of AG-348, considering both the antecedent study and this extension study, unless otherwise specified.

The following endpoints will be analyzed:

Primary:

- Type, incidence, severity, and relationship to study drug of treatment-emergent adverse events (TEAEs); serious adverse events (SAEs); adverse events of special interest (AESIs); and TEAEs leading to dose reduction, treatment interruption, and treatment discontinuation

- Changes from baseline in clinical laboratory tests (ie, serum chemistry, liver function tests [LFTs], hematology, lipids, sex steroids, coagulation, urinalysis), physical examination (PE) findings, bone mineral density T- and Z-scores (total hip, femoral neck, and lumbar spine), vital signs, and 12-lead electrocardiogram (ECG) data

Secondary:

- Cohort 1 only:
  - Proportion of subjects achieving a hemoglobin (Hb) response, defined as a ≥1.5 g/dL (0.93 mmol/L) increase in Hb concentration from baseline that is sustained at 2 or more scheduled assessments at Weeks 16, 20, and 24
  - Average change from baseline in Hb concentration at Weeks 16, 20, and 24
  - Pharmacokinetic endpoints, including plasma concentrations over time and pharmacokinetic parameters of AG-348 (eg, area under the plasma concentration × time curve, maximum [peak] concentration, others as applicable)
  - Exposure-response relationship between safety parameters and AG-348 concentration and relevant AG-348 pharmacokinetic parameters
- All cohorts:
  - Change from baseline in Hb concentration
  - Change from baseline in markers of hemolysis: bilirubin, lactate dehydrogenase (LDH), and haptoglobin levels
  - Change from baseline in markers of erythropoietic activity: reticulocyte percentages
  - Change from baseline in the number of transfusion events
  - Change from baseline in the number of red blood cell (RBC) units transfused
  - Change from baseline in HRQoL PRO scores: Pyruvate Kinase Deficiency Diary (PKDD) and Pyruvate Kinase Deficiency Impact Assessment (PKDIA)

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Methodology:

Overview:

This is a multicenter, open-label, extension study to evaluate the long-term safety, tolerability, and efficacy of treatment with AG-348 in subjects who were previously enrolled in Study AG348-C-006 or Study AG348-C-007.

All subjects enrolled in this extension study, including those who received placebo in Study AG348-C-006, will receive AG-348 during participation in this extension study.

Subjects will be assigned to 1 of the following 3 cohorts, depending on the antecedent study and the previous treatment received in the antecedent study:

- Cohort 1: Subjects who received placebo in Study AG348-C-006
- Cohort 2: Subjects who received AG-348 in Study AG348-C-006

- Cohort 3: Subjects who received AG-348 in Study AG348-C-007

Cohort 1:

Cohort 1 will consist of subjects who received placebo in Study AG348-C-006 and meet the eligibility criteria of this extension study. The first visit of this extension study should coincide with the last visit of Study AG348-C-006. After completion of all scheduled assessments at the subject's last visit of Study AG348-C-006 and before the start of study drug in this extension study, the subject, Investigator, and site personnel will be unblinded to the Study AG348-C-006 treatment allocation of the subject, and the Investigator will determine whether the subject meets all eligibility criteria of this extension study.

The first visit of this extension study should consist of subject consent, screening, confirmation of eligibility, and the start of study drug. If a subject in Cohort 1 cannot start study drug on the first visit of this extension study, discussion with and approval by the Medical Monitor, or designee, will be required for participation in this extension study, and to determine the timing and requirements for the start of study drug and assessments to be performed.

Subjects in Cohort 1 will participate in a 12-week Dose Optimization Period followed by a 12-week Fixed Dose Period. The goal of the Dose Optimization Period is to maximize a subject's increase in Hb while maintaining an acceptable safety profile. After the Dose Optimization Period, each subject will remain on his/her individually optimized dose and enter the Fixed Dose Period.

After completion of the Fixed Dose Period, subjects who, in the opinion of the Investigator, have demonstrated clinical benefit from AG-348 treatment will continue AG-348 treatment in the Continued Treatment Period.

Cohort 2:

Cohort 2 will consist of subjects who received AG-348 in Study AG348-C-006 and meet the eligibility criteria of this extension study. The first visit of this extension study should coincide with the last visit of Study AG348-C-006. After completion of all scheduled assessments at the subject's last visit of Study AG348-C-006 and before the start of study drug in this extension study, the subject, Investigator, and site personnel will be unblinded to the Study AG348-C-006 treatment allocation of the subject, and the Investigator will determine whether the subject meets all eligibility criteria of this extension study.

In Cohort 2, the first visit of this extension study will consist of subject consent, screening, confirmation of eligibility, and the start of study drug on this extension study. Importantly, there will be no planned dosing interruption between the last dose of blinded AG-348 in Study AG348-C-006 and the first dose of open-label AG-348 in this extension study due to the potential for withdrawal hemolysis (an identified risk of AG-348). Specifically, the first dose of open-label AG-348 in this extension study will be administered approximately 12 hours (ie, 12 hours ± 2 hours) after the last dose in Study AG348-C-006. Subjects will continue the AG-348 dose regimen they were receiving at the last visit of Study AG348-C-006 (unless a dose modification is required for reasons related to safety, the subject's dose optimization, or other reasons after discussion with and approval by the Medical Monitor or designee).

Cohort 3:

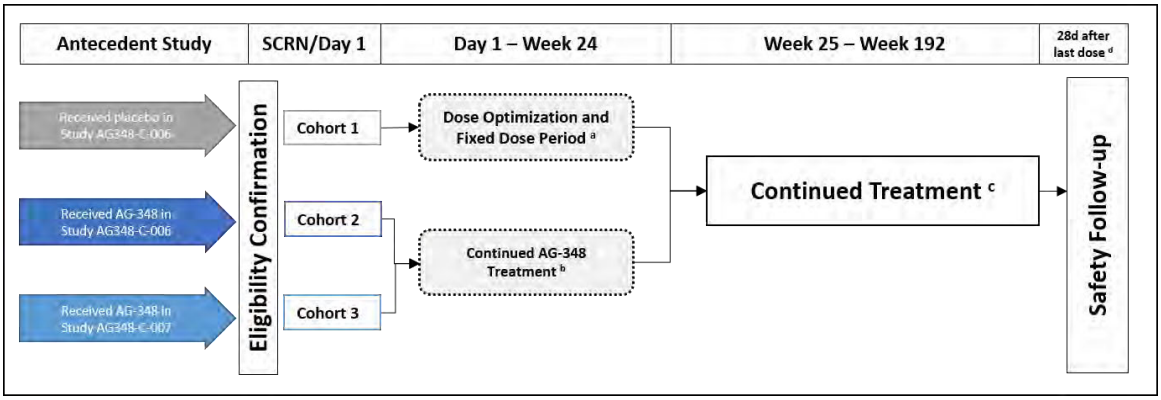
Cohort 3 will consist of subjects who received AG-348 in Study AG348-C-007 and meet the eligibility criteria of this extension study. The first visit of this extension study should coincide with the last visit of Study AG348-C-007. After completion of all scheduled assessments at the subject's last visit of Study AG348-C-007 and before the start of study drug in this extension study, the Investigator will determine whether the subject meets all eligibility criteria of this extension study.

In Cohort 3, the first visit of this extension study will consist of subject consent, screening, confirmation of eligibility, and the start of study drug on this extension study. Importantly, there will

be no planned dosing interruption between the last dose of AG-348 in Study AG348-C-007 and the first dose of AG-348 in this extension study due to the potential for withdrawal hemolysis (an identified risk of AG-348). Specifically, the first dose of AG-348 in this extension study will be administered approximately 12 hours (ie, 12 hours ± 2 hours) after the last dose in Study AG348-C-007. Subjects will continue the AG-348 dose regimen they were receiving at the last visit of Study AG348-C-007 (unless a dose modification is required for reasons related to safety, the subject's dose optimization, or other reasons after discussion with and approval by the Medical Monitor or designee).

Study Schema:

A study schema is provided below.



Abbreviations: d=day; SCRN=Screening.

[a] Eligible subjects in Cohort 1 will initiate treatment with AG-348 in this extension study. Therefore, these subjects will participate in a 12-week Dose Optimization Period followed by a 12-week Fixed Dose Period during the first 24 weeks of this extension study.

[b] Subjects who are in Cohort 2 or 3 will continue AG-348 treatment.

[c] Dosing between Week 24 and Week 25 is continuous.

[d] All subjects who permanently discontinue AG-348 at any time will attend a Safety Follow-up Visit 28 days (±4 days) after the last dose of AG-348 (including the time required to dose taper).

All cohorts discontinuation and follow-up:

All subjects who interrupt or discontinue AG-348 at any time should undergo the recommended dose taper, unless an emergency situation justifies discontinuing or interrupting the study drug abruptly. Whether the dose taper is performed or not, subjects discontinuing or interrupting AG-348 should be monitored for signs of hemolysis and worsening of anemia. All subjects who permanently discontinue AG-348 at any time will attend a Safety Follow-up Visit 28 days (±4 days) after the last dose of AG-348 (including the time required to dose taper).

Subjects with AEs will continue to be followed until resolution of the AE to baseline (ie, baseline of the study during which AE onset occurred), the AE is considered stable within the context of the study, the subject is lost to follow-up, or until 28 days after the last dose of AG-348. All SAEs will be followed until final outcome of the SAE is known or the subject is lost to follow-up.

Number of Subjects Planned:

Approximately 96, with up to 116, subjects are potentially eligible to be enrolled in this extension study: approximately 76 subjects from Study AG348-C-006 and approximately 20, with up to 40, subjects from Study AG348-C-007, if eligible.

Eligibility Criteria:

Inclusion Criteria

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

1. Have provided signed written informed consent prior to participating in this extension study.
2. Have completed the antecedent AG-348 study through the Part 2 Week 24 Visit of Study AG348-C-006 or AG348-C-007.
3. Cohorts 2 and 3: Have demonstrated clinical benefit from AG-348 treatment in the antecedent study, in the opinion of the Investigator.
4. For women of reproductive potential*:
   a. In Cohort 1, have a negative local serum (human chorionic gonadotropin [hCG]) pregnancy test during screening of this extension study.
   b. In Cohort 2 or 3, have a negative local urine pregnancy test during screening of this extension study.

   * 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 screening of this extension study and have an elevated follicle-stimulating hormone [FSH] level indicative of menopause during screening of this extension study or at screening of the antecedent study). **If the result from FSH testing conducted during screening of this extension study is not available on the same day, the woman must have a negative local serum (hCG) or urine pregnancy test during screening and follow contraception requirements (Inclusion Criterion #5) until an elevated FSH result indicative of menopause is confirmed.**
5. 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.
6. Be willing and able to comply with study visits and procedures.

Exclusion Criteria

Subjects who meet any of the following criteria will not be enrolled in this extension study:

1. Have a significant medical condition (including clinically significant laboratory abnormality) that developed during his/her antecedent AG-348 study that confers an unacceptable risk to participating in this extension study, that could confound the interpretation of the study data, and/or that compromises the ability of the subject to complete study visits and procedures.
2. Are currently pregnant or breastfeeding.

3. Have a splenectomy scheduled during the study treatment period.
4. Meet the withdrawal criteria of his/her antecedent AG-348 study during screening of this extension study.

   Withdrawal criteria of the antecedent AG-348 studies are as follows:

   - Withdrawal of consent
   - Development of an intercurrent medical condition that precludes further participation in the study
   - Subject requires use of a prohibited concomitant medication
   - Investigator decision
   - Persistent nonadherence to protocol requirements
   - Pregnancy
   - Lost to follow-up
5. Are currently receiving medications that are strong inhibitors of CYP3A4 that have not been stopped for a duration of at least 5 days or a time frame equivalent to 5 half-lives (whichever is longer) before start of study drug; or strong inducers of CYP3A4 that have not been stopped for a duration of at least 28 days or a time frame equivalent to 5 half-lives (whichever is longer) before start of study drug on this extension study.
6. Have received anabolic steroids, including testosterone preparations, within 28 days prior to start of study drug on this extension study.
7. Have received hematopoietic stimulating agents (eg, erythropoietins, granulocyte colony stimulating factors, thrombopoietins) within 28 days prior to start of study drug on this extension study.
8. Have exposure to any investigational drug other than AG-348, device, or procedure within 3 months prior to start of study drug on this extension study.

Investigational Product, Dosage, and Mode of Administration:

AG-348 will be administered orally as tablets of different sizes for the 5, 20, and 50 mg dose levels. Doses of AG-348 may be taken with or without food. Tablets should be swallowed whole with water and not crushed, chewed, or dissolved in water. The dose regimen will be 5, 20, or 50 mg twice daily (BID), with each dose administered approximately 12 hours (ie, 12 hours ± 2 hours) apart, unless a dose taper is required at any time during this extension study, in which case the frequency of administration will be once daily (QD) or once every other day (QOD) during different steps of the taper. At any time during this extension study, the Investigator can reduce the subject's dose or interrupt dosing for reasons related to safety.

For subjects in Cohort 1, the initial dose will be 5 mg BID with the potential for 2 sequential dose level increases (from 5 mg BID to 20 mg BID and from 20 mg BID to 50 mg BID), which may occur at the Week 4 and/or Week 8 Visits.

Subjects in Cohorts 2 and 3 will continue the dose regimen they were receiving at their last visit of Study AG348-C-006 or AG348-C-007, respectively (unless otherwise noted).

Duration of Treatment and End of Study:

Subjects may continue AG-348 treatment for up to a maximum of 192 weeks in this extension study (not including the time required for completion of the recommended dose taper) until they meet study withdrawal criteria or the study is closed.

This extension study will end when all subjects have discontinued or completed the study, are lost to follow-up, or the Study Sponsor terminates the study.

Reference Therapy, Dosage and Mode of Administration:

Not applicable, as this is an open-label study in which all subjects receive AG-348.

Criteria for Evaluation (Measurements):

Safety:

Results of clinical laboratory tests over time (ie, serum chemistry, LFT, hematology, lipids, sex steroids, urinalysis, coagulation); PE findings; vital signs; ECG intervals; and bone mineral density (total hip, femoral neck, and lumbar spine); AEs; menstrual diary

Efficacy:

Hemoglobin, haptoglobin, bilirubin, LDH, reticulocyte percentages, EPO, erythroferrone, and soluble transferrin receptor

Number of transfusion events and number of RBC units transfused

Pharmacokinetics:

AG-348 plasma concentrations (*Cohort 1 only*)

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HRQoL:

PKDD and PKDIA

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Statistical Methods:

Analysis Sets:

- Safety Analysis Set (SAS) will be defined as all subjects who are exposed to any amount of study drug in this extension study.

Statistical Methods:

All analyses will be based on the SAS unless otherwise specified in the detailed Statistical Analysis Plan (SAP). Statistical analysis details will be provided in the SAP.

Efficacy:

The number and percentage of subjects experiencing an Hb response will be summarized for Cohort 1 subjects in the SAS based on the extension study assessments. If less than 2 out of the targeted 3 assessments are available, the subject will be considered a non-responder. Hemoglobin values assessed within 2 months (61 days) of a transfusion will be excluded from the analysis.

For Hb, markers of hemolysis, markers of erythropoietic activity, and markers of iron disposition and/or metabolism, a summary of their actual values and change from baseline at each visit will be provided for all cohorts.

The number of transfusion events and number of RBC units transfused will be summarized and adjusted for the duration on study drug. Additional modeling may be planned and described in detail in the SAP.

For PKDD and PKDIA, summary statistics will be provided based on the validated algorithm once available from Study AG348-C-006.

Safety:

For safety analyses, only descriptive analyses will be performed (ie, no formal statistical testing will be performed). Summaries of TEAEs using the number and percentages of subjects as well as the number of events per 100 patient-years (number of events adjusted for the total duration of exposure) will be provided. Similar analyses may be provided for SAEs, AESIs, and AEs leading to study drug discontinuation, interruption, or dose reduction, as applicable. Summaries of clinical laboratory results, bone mineral density T- and Z-scores, vital signs, and ECG, including their actual values and change from baseline values at each visit, will be provided.

Pharmacokinetics:

For Cohort 1, the plasma pharmacokinetic parameters of AG-348 will be computed using noncompartmental methods based on observed plasma concentrations of the parent and actual sample collection times. Descriptive statistics (eg, number of subjects [n], mean, standard deviation [SD], coefficient of variation, median, minimum and maximum, geometric mean, geometric coefficient of variation) will be used to summarize the pharmacokinetic parameters for AG-348.

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

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4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
2,3-DPG	2,3-diphosphoglycerate
ADP	Adenosine diphosphate
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
AUC	Area under the plasma concentration × time curve
AUC_{0-12}	Area under the plasma concentration × time curve from 0 to 12 hours
AUC_{0-last}	Area under the plasma concentration × time curve from time 0 to the time of the last measurable concentration
BID	Twice daily
BP	Blood pressure
C_{max}	Maximum (peak) concentration
CTCAE	Common Terminology Criteria for Adverse Events
CYP	Cytochrome P450
DXA	Dual-energy X-ray absorptiometry
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EPO	Erythropoietin
EU	European Union
FDA	Food and Drug Administration
FSH	Follicle-stimulating hormone
GCP	Good Clinical Practice
Hb	Hemoglobin
hCG	Human chorionic gonadotropin
HRQoL	Health-related quality of life
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Conference on Harmonization

Abbreviation	Definition
IEC	Independent Ethics Committee
IMP	Investigational medicinal product
IRB	Institutional Review Board
LDH	Lactate dehydrogenase
LFTs	Liver function tests
CCI	[redacted]
MAD	Multiple ascending dose study
MedDRA	Medical Dictionary for Regulatory Activities
CCI	[redacted]
NHS	Natural history study
PD	Pharmacodynamics
PE	Physical examination
PEP	Phosphoenolpyruvate
P-gp	P-glycoprotein
PK	Pyruvate kinase
PKDD	Pyruvate Kinase Deficiency Diary
PKDIA	Pyruvate Kinase Deficiency Impact Assessment
PKL	Liver-specific form of pyruvate kinase
PKM	Muscle-specific form of pyruvate kinase
PKR	RBC-specific form of pyruvate kinase
PRO	Patient-reported outcome
PT	Preferred Term
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
RBC	Red blood cell
SAD	Single ascending dose study
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS	Safety Analysis Set
SD	Standard deviation

Abbreviation	Definition
SOC	System Organ Class
TEAE	Treatment-emergent adverse event
T_{max}	Time to maximum (peak) concentration
TT	Transfusion trigger
UGT	Uridine 5′-diphospho-glucuronosyltransferase
ULN	Upper limit of normal
US	United States
WT	Wild-type

5. INTRODUCTION

5.1. Pyruvate Kinase Deficiency

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

5.1.1. Epidemiology and Prevalence

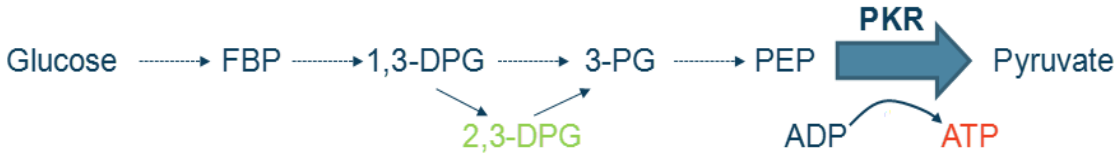
Epidemiological data for PK deficiency are scarce; however, the current estimated diagnosed prevalence of patients with PK deficiency in the US and EU5 (ie, France, Germany, Italy, Spain, the United Kingdom) is approximately 2,400 cases (Carey et al, 2000; de Medicis et al, 1992). As with many rare genetic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

5.1.2. Biochemistry and Genetics

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

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

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



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

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

A non-drug study protocol to obtain critical information regarding the natural history of PK deficiency and the range and incidence of related symptoms, treatments, and complications—known as the PK Deficiency Natural History Study (NHS) (NCT02053480, 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 only identified as a consequence of the systematic genotyping effort for participants in this NHS. This highlights the evolving understanding of the genetic basis of PK deficiency.

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

5.1.3. Clinical Characteristics

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

thus, pigmented gallstones are common in both children and adults with the disease. Additionally, iron overload is progressive and can ultimately lead to life-threatening symptoms.

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

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

5.2. Investigational Product (AG-348)

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

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.

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

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

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

5.2.3. Summary of AG-348 Clinical Data

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

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

A brief overview of AG-348 pharmacokinetic and PD data is provided in Section 5.2.3.1 and Section 5.2.3.2, respectively. An overview of the available safety from these studies as well as

preliminary efficacy results from the DRIVE-PK study is included in Section 5.2.3.3 and Section 5.2.3.4, respectively.

Please refer to the AG-348 IB for additional details on all clinical studies and results.

5.2.3.1. Summary of AG-348 Pharmacokinetics

The pharmacokinetic profile of AG-348 has been well characterized in the Phase 1 single-ascending dose (SAD) study (AG348-C-001) and Phase 1 multiple-ascending dose (MAD) study (AG348-C-002), conducted in healthy adult subjects. The pharmacokinetics of AG-348 increased in a dose-proportional manner across tested doses in the SAD study and at lower doses in the MAD study. CCI [redacted] The effective half-life of AG-348 has been estimated to be approximately 3 to 6 hours.

A capsule formulation was used in the SAD and MAD studies and the DRIVE-PK study. A tablet formulation has subsequently been introduced into DRIVE-PK, and is being used in all ongoing studies. Before 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 after the final dose of AG-348. In the SAD study, after a single dose of AG-348, a minimal increase in the concentration of ATP was observed at 24 to 120 hours postdose. In contrast to the SAD study, significant increases in ATP were observed in the MAD study, and the concentration of ATP remained elevated through 120 hours after the final dose of AG-348.

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

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

5.2.3.3. Summary of AG-348 Clinical Safety Data

Overall, AG-348 has been generally well tolerated among healthy adult subjects and adult subjects with PK deficiency. Important identified risks associated with administration of AG-348 in clinical studies include CCI withdrawal hemolysis, and insomnia (not clinically serious [ie, not Grade 3 or Grade 4]).

Potential risks of AG-348 administration include anaphylactoid reaction, CCI, gastrointestinal disturbances, CCI and triglyceride increase. CCI Please refer to Section 11.2.6 for additional information 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

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.

As mentioned in Section 5.1, in patients with PK deficiency, RBCs and their progenitors are characterized by changes in metabolism associated with defective glycolysis. AG-348 is an activator of WT and several mutant forms of PKR with the potential to correct the underlying pathology of PK deficiency by activating PKR and increasing glycolytic pathway activity in RBCs to reduce hemolysis.

To evaluate the efficacy and safety of AG-348 across the disease spectrum of PK deficiency, 2 pivotal studies investigating the treatment of AG-348 in subjects with PK deficiency have been initiated. Study AG348-C-006 is a randomized, double-blind, placebo-controlled study being conducted in adult subjects with PK deficiency who are not regularly receiving transfusions, while a separate open-label study (Study AG348-C-007) is being conducted in adult subjects with PK deficiency who are regularly receiving transfusions.

5.3.1. Purpose of the Study

This study (Study AG348-C-011) is a multicenter, open-label extension study to evaluate the long-term safety, tolerability, and efficacy of treatment with AG-348 in subjects who were previously enrolled in Study AG348-C-006 or Study AG348-C-007.

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, iron overload, susceptibility to infections after splenectomy, worsening anemia during pregnancy, and symptoms associated with chronic hemolytic anemia (Rider et al, 2011).

Based on the pathophysiology of PK deficiency and the mechanism of action of AG-348, it is assumed that chronic, life-long treatment with AG-348 will be required to maintain clinical benefit. The long-term data collected during the course of this extension study will inform the long-term benefit/risk profile of chronic AG-348 treatment in this patient population.

5.3.2. Justification of the Study Design

This is a 3-cohort, multicenter, open-label extension study in which all subjects will receive treatment with AG-348. As this study will include subjects who received AG-348 in 1 of the 2 pivotal trials (ie, AG348-C-006 or AG348-C-007) and subjects who previously received placebo in the AG348-C-006 study, this extension study will consist of 3 cohorts as follows:

- Cohort 1: Subjects who received placebo in Study AG348-C-006
- Cohort 2: Subjects who received AG-348 in Study AG348-C-006
- Cohort 3: Subjects who received AG-348 in Study AG348-C-007

Subjects who previously received placebo on Study AG348-C-006 (ie, Cohort 1), will have the chance to receive initial treatment with AG-348 in this study. As subjects in Cohort 1 will be initiating treatment with AG-348, they will follow the same dose scheme of Study AG348-C-006 with a 12-week Dose Optimization Period to identify their individually optimized dose of AG-348 followed by a 12-week Fixed Dose Period before entering the long-term Continued Treatment Period.

Subjects who received AG-348 on Studies AG348-C-006 or AG348-C-007 (ie, Cohort 2 and Cohort 3, respectively) will be able to continue receiving treatment with AG-348 during this extension study. Because withdrawal hemolysis is an identified risk of AG-348, it is critical that subjects in Cohorts 2 and 3, who were actively receiving treatment with AG-348, maintain treatment without dose interruption when transitioning to this extension study. Therefore, the last visit of the antecedent study should coincide with the first visit of this extension study to avoid interruption in study drug administration.

5.3.3. Justification for Individual Dose Optimization for Subjects in Cohort 1

This extension study will have an individual Dose Optimization Period for subjects in Cohort 1 similar to the individual dose optimization performed in Study AG348-C-006.

The reason for performing individual dose optimization is based on prior experience from the Phase 2 DRIVE-PK study (Study AG348-C-003). In Study AG348-C-003, subjects with PK deficiency were randomized to receive AG-348 at a dose of either 50 mg BID or 300 mg BID.

However several subjects required dose reductions during the study either due to excess increase in Hb or the occurrence of AEs, such as insomnia, headache, or nausea. Therefore, the actual doses that were evaluated in Study AG348-C-003 ranged from 5 mg once daily (QD) to 300 mg BID. In addition, an evaluation of the efficacy endpoint in Study AG348-C-003 showed that subjects responded to AG-348 across this wide range of doses (5 mg QD to 300 mg BID). Given these observations combined with the number of mutations that are known to occur in patients with PK deficiency, individual dose optimization is incorporated in Cohort 1, allowing each subject to gradually increase his or her dose of AG-348 in order to identify a dose that confers maximum benefit with minimum risk to that subject.

Preliminary data from the DRIVE-PK study indicated that subjects with PK deficiency who respond to activation of the PKR protein typically do so within 2-3 weeks. Therefore, every 4 weeks during Part 1, subjects in Cohort 1 are assessed for safety and efficacy (as defined by Hb increase) to determine if their dose should be increased, maintained at the current level, or decreased. After identification of an optimized dose, each subject is then maintained on AG-348 at their optimized dose for a fixed period of time (Fixed Dose Period) to allow for efficacy evaluation.

As in Study AG348-C-006, all subjects in Cohort 1 of this extension study (Study AG348-C-011) will start on an initial AG-348 dose of 5 mg BID with 2 potential sequential steps for dose level increases (ie, from 5 mg BID to 20 mg BID and from 20 mg BID to 50 mg BID), depending on safety and Hb change. Similar to Study AG348-C-006, subjects in Cohort 1 will be assessed every 4 weeks during Part 1 for safety and efficacy (as defined by Hb increase) to determine if their dose should be increased, maintained at the current level, or decreased. The rationale for the dose levels being used in the Dose Optimization Period for Cohort 1 (5 mg, 20 mg, and 50 mg) is provided in Section 5.3.4.

5.3.4. Rationale for the Doses Selected for the Individual Dose Optimization

The dose levels being used in the Dose Optimization Period for Cohort 1 (5 mg, 20 mg, and 50 mg) in this extension study are the same as those used in the antecedent studies (Study AG348-C-006 and Study AG348-C-007).

To assist with dose selection, an exposure-response analysis was conducted using pharmacokinetic, efficacy, and safety data from the DRIVE-PK study. Briefly, a sequential population pharmacokinetic-efficacy model was developed using increase in Hb as the efficacy endpoint, while a binary logistic regression approach incorporating the safety endpoints of ALT, 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.

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doses of 5 mg BID, 20 mg BID, and 50 mg BID were selected for the Dose Optimization Period in Studies AG348-C-006 and AG348-C-007. These same doses will be used in the Dose Optimization Period for subjects in Cohort 1 of this extension study.

Subjects in Cohort 2 and Cohort 3 will have undergone a Dose Optimization Period in the antecedent study, and therefore, will continue to receive the same dose in this extension study as they were receiving at their last visit in the antecedent study (unless otherwise noted in Section 7.1.2 or Section 7.1.3, respectively).

6. STUDY OBJECTIVES AND ENDPOINTS

6.1. Study Objectives

6.1.1. Primary Objective

The primary objective of the study is to evaluate the long-term safety and tolerability of AG-348.

6.1.2. Secondary Objectives

Secondary objectives of the study are as follows:

- To evaluate the long-term efficacy of AG-348
- To evaluate the efficacy of AG-348 in increasing Hb concentrations in subjects who previously received placebo in Study AG348-C-006 (*Cohort 1 only*)
- To determine the effect of AG-348 on health-related quality of life (HRQoL) using patient reported outcomes (PROs)
- To evaluate the pharmacokinetics of AG-348 after oral administration (*Cohort 1 only*)
- To evaluate the relationship between AG-348 pharmacokinetics and safety parameters (*Cohort 1 only*)

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[redacted]

6.2. Study Endpoints

The baseline value is defined as the most recent measurement(s) before the first dose of AG-348, considering both the antecedent study and this extension study, unless otherwise specified in Section 12.2.1.

6.2.1. Primary Endpoint

The primary endpoint is to assess the long-term safety and tolerability of AG-348 by:

- Type, incidence, severity, and relationship to study drug of treatment-emergent adverse events (TEAEs); serious adverse events (SAEs); AESIs; and TEAEs leading to dose reduction, treatment interruption, and treatment discontinuation
- Changes from baseline in clinical laboratory tests (ie, serum chemistry, liver function tests [LFTs], hematology, lipids, sex steroids, coagulation, urinalysis), physical examination (PE) findings, bone mineral density T- and Z-scores (total hip, femoral neck, and lumbar spine), vital signs, and 12-lead electrocardiogram (ECG) data

6.2.2. Secondary Endpoints

The secondary endpoints of the study are as follows:

- Cohort 1 only:
  - Proportion of subjects achieving a hemoglobin response, defined as a ≥1.5 g/dL (0.93 mmol/L) increase in Hb concentration from baseline that is sustained at 2 or more scheduled assessments at Weeks 16, 20, and 24
  - Average change from baseline in Hb concentration at Weeks 16, 20, and 24
  - Pharmacokinetic endpoints, including plasma concentrations over time and pharmacokinetic parameters of AG-348 (eg, AUC, C_{max}, others as applicable)
  - Exposure-response relationship between safety parameters and AG-348 concentration and relevant AG-348 pharmacokinetic parameters
- All cohorts:
  - Change from baseline in Hb concentration
  - Change from baseline in markers of hemolysis: bilirubin, lactate dehydrogenase (LDH), and haptoglobin levels
  - Change from baseline in markers of erythropoietic activity: reticulocyte percentages
  - Change from baseline in the number of transfusion events
  - Change from baseline in the number of RBC units transfused
  - Change from baseline in HRQoL PRO scores: Pyruvate Kinase Deficiency Diary (PKDD) and Pyruvate Kinase Deficiency Impact Assessment (PKDIA)


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7. STUDY DESIGN

7.1. Study Design Overview

This is a multicenter, open-label, extension study to evaluate the long-term safety, tolerability, and efficacy of treatment with AG-348 in subjects who were previously enrolled in Study AG348-C-006 or Study AG348-C-007.

Subjects will be assigned to 1 of the following 3 cohorts, depending on the antecedent study and the previous treatment received in the antecedent study:

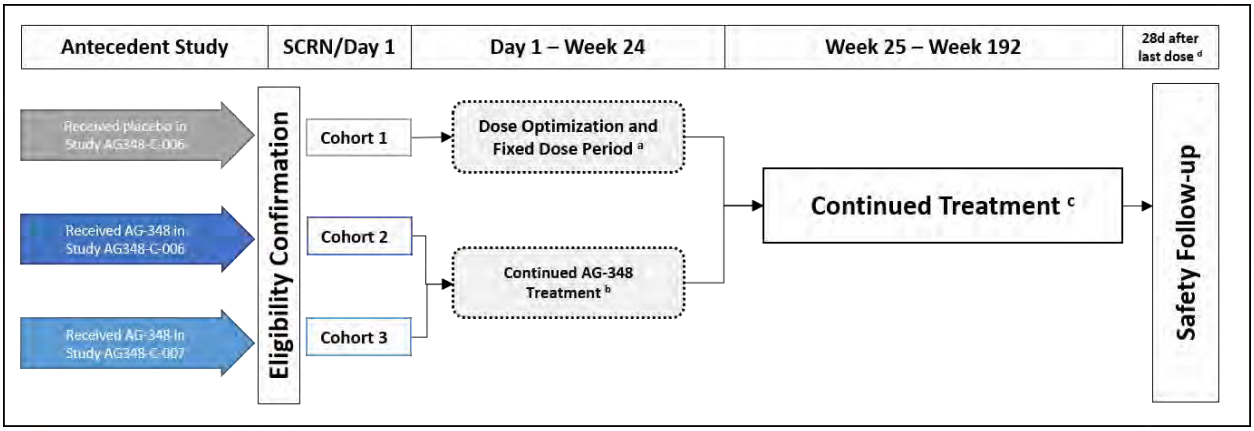
- Cohort 1: Subjects who received placebo in Study AG348-C-006 (Section 7.1.1)
- Cohort 2: Subjects who received AG-348 in Study AG348-C-006 (Section 7.1.2)
- Cohort 3: Subjects who received AG-348 in Study AG348-C-007 (Section 7.1.3)

All subjects enrolled in this extension study, including those who received placebo in Study AG348-C-006, will receive AG-348 during participation in this extension study. Subjects may continue AG-348 treatment for up to a maximum of 192 weeks in this extension study (not including the time required for completion of the recommended dose taper) until they meet study withdrawal criteria (Section 8.3) or the study is closed (Section 7.5).

At any time during the study, the Investigator can discontinue, interrupt, or reduce the subject's dose of study drug for reasons related to safety (as described in Section 9.3).

An overview of the study design is provided in Figure 2. Details about screening, the Dose Optimization and Fixed Dose Periods (Cohort 1 only), the Continued Treatment Period, and Discontinuation and Safety Follow-up are described in Section 7.2.

Figure 2: Overview of Design for Study AG348-C-011



Abbreviations: d=day; SCRN=Screening.

[a] Eligible subjects in Cohort 1 will initiate treatment with AG-348 in this extension study. Therefore, these subjects will participate in a 12-week Dose Optimization Period followed by a 12-week Fixed Dose Period during the first 24 weeks of this extension study.

[b] Subjects who are in Cohort 2 or 3 will continue AG-348 treatment.

[c] Dosing between Week 24 and Week 25 is continuous.

[d] All subjects who permanently discontinue AG-348 at any time will attend a Safety Follow-up Visit 28 days (±4 days) after the last dose of AG-348 (including the time required to dose taper).

7.1.1. Cohort 1

Cohort 1 will consist of subjects who received placebo in Study AG348-C-006 and meet the eligibility criteria of this extension study. The first visit of this extension study should coincide with the last visit of Study AG348-C-006. After completion of all scheduled assessments at the subject's last visit of Study AG348-C-006 and before the start of study drug in this extension study, the subject, Investigator, and site personnel will be unblinded to the Study AG348-C-006 treatment allocation of the subject, and the Investigator will determine whether the subject meets all eligibility criteria of this extension study.

The first visit of this extension study should consist of subject consent, screening, confirmation of eligibility, and the start of study drug. If a subject in Cohort 1 cannot start study drug on the first visit of this extension study, discussion with and approval by the Medical Monitor, or designee, will be required for participation in this extension study, and to determine the timing and requirements for the start of study drug and assessments to be performed.

Subjects in Cohort 1 will participate in a 12-week Dose Optimization Period followed by a 12-week Fixed Dose Period (see Section 7.2.2 and Section 7.2.3, respectively). The goal of the Dose Optimization Period is to maximize a subject's increase in Hb while maintaining an acceptable safety profile. After the Dose Optimization Period, each subject will remain on his/her individually optimized dose and enter the Fixed Dose Period. A schedule of assessments is provided for the Dose Optimization Period and Fixed Dose Period for Cohort 1 in Table 4.

After completion of the Fixed Dose Period, subjects who, in the opinion of the Investigator, have demonstrated clinical benefit from AG-348 treatment will continue AG-348 treatment in the Continued Treatment Period (see Section 7.2.4).

7.1.2. Cohort 2

Cohort 2 will consist of subjects who received AG-348 in Study AG348-C-006 and meet the eligibility criteria of this extension study. The first visit of this extension study should coincide with the last visit of Study AG348-C-006. After completion of all scheduled assessments at the subject's last visit of Study AG348-C-006 and before the start of study drug in this extension study, the subject, Investigator, and site personnel will be unblinded to the Study AG348-C-006 treatment allocation of the subject, and the Investigator will determine whether the subject meets all eligibility criteria of this extension study.

In Cohort 2, the first visit of this extension study will consist of subject consent, screening, confirmation of eligibility, and the start of study drug on this extension study. Importantly, there will be no planned dosing interruption between the last dose of blinded AG-348 in Study AG348-C-006 and the first dose of open-label AG-348 in this extension study due to the potential for withdrawal hemolysis (an identified risk of AG-348). Specifically, the first dose of open-label AG-348 in this extension study will be administered approximately 12 hours (ie, 12 hours ± 2 hours) after the last dose in Study AG348-C-006. Subjects will continue the AG-348 dose regimen they were receiving at the last visit of Study AG348-C-006 (unless a dose modification is required for reasons related to safety, the subject's dose optimization, or other reasons after discussion with and approval by the Medical Monitor or designee).

A schedule of assessments for the first 24 weeks of Cohort 2 is provided in Table 5. Additional details regarding the Continued Treatment Period are provided in Section 7.2.4.

7.1.3. Cohort 3

Cohort 3 will consist of subjects who received AG-348 in Study AG348-C-007 and meet the eligibility criteria of this extension study. The first visit of this extension study should coincide with the last visit of Study AG348-C-007. After completion of all scheduled assessments at the subject's last visit of Study AG348-C-007 and before the start of study drug in this extension study, the Investigator will determine whether the subject meets all eligibility criteria of this extension study.

In Cohort 3, the first visit of this extension study will consist of subject consent, screening, confirmation of eligibility, and the start of study drug on this extension study. Importantly, there will be no planned dosing interruption between the last dose of AG-348 in Study AG348-C-007 and the first dose of AG-348 in this extension study due to the potential for withdrawal hemolysis (an identified risk of AG-348). Specifically, the first dose of AG-348 in this extension study will be administered approximately 12 hours (ie, 12 hours ± 2 hours) after the last dose in Study AG348-C-007. Subjects will continue the AG-348 dose regimen they were receiving at the last visit of Study AG348-C-007 (unless a dose modification is required for reasons related to safety, the subject's dose optimization, or other reasons after discussion with and approval by the Medical Monitor or designee).

A schedule of assessments for the first 24 weeks of Cohort 3 is provided in Table 5. Additional details regarding the Continued Treatment Period are provided in Section 7.2.4.

Subjects in Cohort 3 should continue to be transfused with their mean number of blood units when they reach their Individual transfusion trigger (TT). As defined in Study AG348-C-007, the Individual TT is:

- The sum of the Hb values within 1 week before and closest to each transfusion (when available) for the transfusions in the 52-week period before informed consent (for Study AG348-C-007) divided by the number of such available Hb values 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.
- The mean number of blood units is the sum of the number of units transfused at each transfusion during the 52-week period before informed consent (for Study AG348-C-007) divided by the number of transfusions during this period. Fractional numbers will be rounded to the closest integer.

At the discretion of the Investigator, some subjects may need additional hematology assessments. Blood sampling for these additional assessments (unscheduled visits) should be analyzed by the central laboratory but 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 9.5.4.

7.2. Study Description

7.2.1. Screening Period

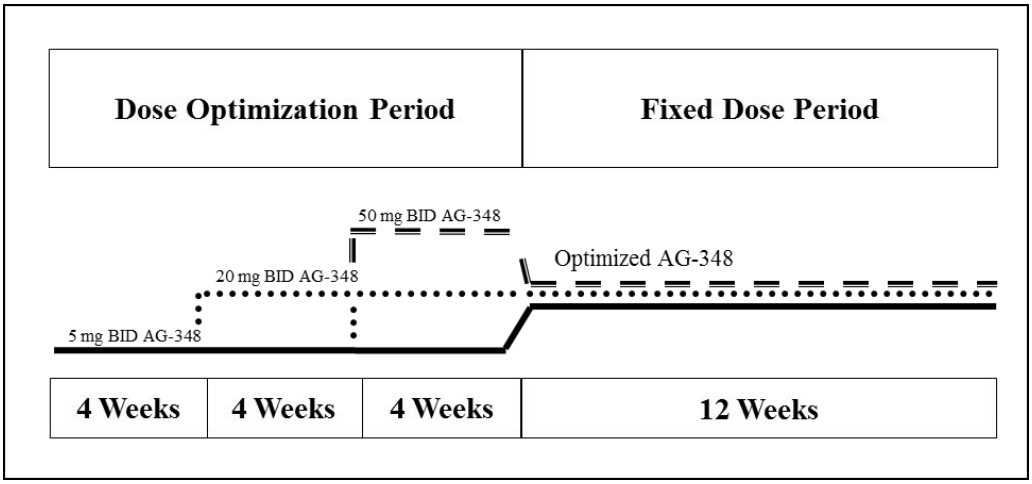
A subject's screening during this extension study should coincide with his/her last visit of their antecedent study (ie, Study AG348-C-006 or Study AG348-C-007). Assessments that overlap between the last visit of the antecedent study and screening of this extension study only need to be performed once.

All assessments for eligibility for this extension study must be completed before a subject can begin study drug in this extension study.

7.2.2. Dose Optimization Period (Cohort 1 Only)

The Dose Optimization and Fixed Dose Periods of Cohort 1 will replicate the Dose Optimization and Fixed Dose Periods of Study AG348-C-006 in terms of frequency of assessments (Table 4) and dosing schema (Figure 3).

Figure 3: Cohort 1 Dosing Schema for the First 24 Weeks, Study AG348-C-011



Abbreviations: BID=twice daily.

Subjects in Cohort 1 will participate in a 12-week Dose Optimization Period followed by a 12-week Fixed Dose Period. The Dose Optimization Period will begin with the first dose of AG-348 administered at the study site.

The starting dose will be 5 mg BID, with 2 potential sequential steps for dose level increases (from 5 mg BID to 20 mg BID and from 20 mg BID to 50 mg BID; no increases beyond 50 mg BID will be allowed) at the Week 4 and/or Week 8 Visits. Subjects will be assessed for safety and efficacy (as defined by Hb increase) every 4 weeks during the Dose Optimization Period to determine if their dose should be increased, maintained at the current level, or decreased. At the Week 4 and Week 8 Visits, AG-348 dose should be increased to the next dose level if the subject has met both of the following criteria:

- The subject is tolerating the study drug **and**

- The subject's Hb concentrations on the day of the visit based on local laboratory results is lower than 2.5 g/dL (1.55 mmol/L) below the ULN as applies to men and women.

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

At the Week 12 Visit, if the subject has tolerated the study drug, then the subject will remain at his/her current dose level. 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, 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.

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.

A schedule of assessments is provided for the Dose Optimization Period in Table 4.

7.2.3. Fixed Dose Period (Cohort 1 Only)

The Fixed Dose Period is a 12-week period after the Week 12 Visit through Week 24.

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

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

A schedule of assessments is provided for the Fixed Dose Period in Table 4.

7.2.4. Continued Treatment Period

After the Week 24 Visit, subjects in Cohort 1 who, in the opinion of the Investigator, have demonstrated clinical benefit from AG-348 treatment will begin the Continued Treatment Period. During this period, they will continue the AG-348 dose regimen they were receiving at the Week 24 Visit (unless a dose modification is required for reasons related to safety, the subject's dose optimization, or other reasons after discussion with and approval by the Medical Monitor or designee).

Subjects in Cohorts 2 and 3 will continue treatment with AG-348 in this extension study as noted in Section 7.1. A schedule of assessments for the first 24 weeks of Cohorts 2 and 3 is provided in Table 5.

A schedule of assessments for the Continued Treatment Period after Week 24 for all cohorts is provided in Table 6.

7.2.5. Discontinuation and Safety Follow-up

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

All subjects who permanently discontinue AG-348 at any time will attend a Safety Follow-up Visit 28 days (±4 days) after the last dose of AG-348 (including the time required to dose taper).

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

7.3. Number of Subjects to be Enrolled

Approximately 96, with up to 116, subjects are potentially eligible to be enrolled in this extension study: approximately 76 subjects from Study AG348-C-006 and approximately 20, with up to 40, subjects from Study AG348-C-007, if eligible.

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

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

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

Documented approval from the Sponsor is required before these modifications can be implemented.

7.4.1. Allowable Modifications

The following modifications are allowed in the event of a public health emergency or natural disaster and must be reported as protocol deviations; refer to Section 10.1 for the timing of assessments:

- Alternative distribution of study drug
  - A 3-month supply of AG-348 may be shipped to a local health care provider or pharmacy or, if necessary, directly to a subject. Delivery of a greater than

3-month supply of AG-348 must be reviewed and approved in advance by the Sponsor (or representative), in agreement with the investigator.

  - Secure, trackable delivery methods (delivery service companies [eg, DHL], couriers, and hand delivery) must be used.
  - Sponsor (or representative) approval is required before each shipment. Shipment will be permitted only if, at minimum, a telemedicine visit has been conducted that incorporates appropriate safety assessments.

- Returning unused study drug and empty study drug packaging
  - Return of unused study drug and empty study drug packaging may be delayed until the subject's next visit to the study site. In certain circumstances, the nature of the return process may vary (eg, personal protective equipment may be required).
- Telemedicine visits
  - Telemedicine visits, preferably via video conference, are permissible for all assessments that can be completed via this mode (eg, medical history, concomitant medications, review of AEs).
- Use of laboratories and health care providers not specified in the clinical trial documentation
  - For assessments that cannot be completed via telemedicine, the use of health care providers and laboratories that are not specified in the clinical trial documentation (such as an imaging facility, clinic, or local practice that is more readily accessible by the subject) is permissible for all assessments that can be completed via this mode (eg, blood collection for laboratory assessments, ECG, physical examinations, imaging). The use of noncentral imaging (dual-energy X-ray absorptiometry [DXA] CCI [redacted] requires prior agreement with Sponsor (or representative).
  - Use of a laboratory or health care provider not specified in the clinical trial documentation requires coordination between the subject, the investigator, and the subject's local health care provider.
  - The investigator must document their review of the results provided by laboratories and health care providers not specified in the clinical trial documentation.
- Home health study support
  - For assessments that cannot be completed via telemedicine, home health nursing visits are permissible for all assessments that can be completed via this mode (eg, physical examination, collection of laboratory samples).
  - The Sponsor may facilitate and coordinate these visits with the study site.

  - The Investigator must document their review of the results of home health nursing visits.
- Virtual informed consent/reconsent in lieu of in-person informed consent/reconsent
  - Consent to participate in this study may be completed virtually and documented in the relevant subject medical records because subjects are entering this study after participating in a previous study of the same study drug and study visits/ assessments in this study will be conducted at the same or a lower frequency than in the preceding study.
  - Reconsent (ie, consenting to an amended version of the protocol) may be completed virtually and documented in the relevant subject medical records.
  - The other allowable modifications described in this section may require consent from the subject because their implementation requires a variation from the specifications in the protocol to which the subject has consented (eg, consent for a home visit, consent to provide name and address to a third-party delivery service, consent to a new mode of completing study procedures and receiving study drug). In these instances, consent may be completed virtually and documented in the relevant subject medical records.

7.5. Criteria for Study Closure

This study may be 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 AG-348
- Decisions of competent authorities or Institutional Review Board (IRB)/Independent Ethics Committee (IEC)
- Other administrative or business reasons

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

8. STUDY POPULATION

8.1. Inclusion Criteria

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

1. Have provided signed written informed consent prior to participating in this extension study.
2. Have completed the antecedent AG-348 study through the Part 2 Week 24 Visit of Study AG348-C-006 or AG348-C-007.
3. Cohorts 2 and 3: Have demonstrated clinical benefit from AG-348 treatment in the antecedent study, in the opinion of the Investigator.
4. For women of reproductive potential*:
   a. In Cohort 1, have a negative local serum (human chorionic gonadotropin [hCG]) pregnancy test during screening of this extension study.
   b. In Cohort 2 or 3, have a negative local urine pregnancy test during screening of this extension study.

   * 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 screening of this extension study and have an elevated follicle-stimulating hormone [FSH] level indicative of menopause during screening of this extension study or at screening of the antecedent study). If the result from FSH testing conducted during screening of this extension study is not available on the same day, the woman must have a negative local serum (hCG) or urine pregnancy test during screening and follow contraception requirements (Inclusion Criterion #5) until an elevated FSH result indicative of menopause is confirmed.
5. 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.

6. Be willing and able to comply with study visits and procedures.

8.2. Exclusion Criteria

Subjects who meet any of the following criteria will not be enrolled in this extension study:

1. Have a significant medical condition (including clinically significant laboratory abnormality) that developed during his/her antecedent AG-348 study that confers an unacceptable risk to participating in this extension study, that could confound the interpretation of the study data, and/or that compromises the ability of the subject to complete study visits and procedures.
2. Are currently pregnant or breastfeeding.
3. Have a splenectomy scheduled during the study treatment period.
4. Meet the withdrawal criteria of his/her antecedent AG-348 study during screening of this extension study.

   Withdrawal criteria of the antecedent AG-348 studies are as follows:

   - Withdrawal of consent
   - Development of an intercurrent medical condition that precludes further participation in the study
   - Subject requires use of a prohibited concomitant medication
   - Investigator decision
   - Persistent nonadherence to protocol requirements
   - Pregnancy
   - Lost to follow-up
5. Are currently receiving medications that are strong inhibitors of CYP3A4 that have not been stopped for a duration of at least 5 days or a time frame equivalent to 5 half-lives (whichever is longer) before start of study drug; or strong inducers of CYP3A4 that have not been stopped for a duration of at least 28 days or a time frame equivalent to 5 half-lives (whichever is longer) before start of study drug on this extension study.
6. Have received anabolic steroids, including testosterone preparations, within 28 days prior to start of study drug on this extension study.
7. Have received hematopoietic stimulating agents (eg, erythropoietins, granulocyte colony stimulating factors, thrombopoietins) within 28 days prior to start of study drug on this extension study.
8. Have exposure to any investigational drug other than AG-348, device, or procedure within 3 months prior to start of study drug on this extension study.

8.3. Subject Withdrawal Criteria

Subjects have the right to withdraw from the study drug or the study at any time for any reason. A subject's withdrawal from study drug or the study will not jeopardize the relationship with their health care providers or affect their future care. 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 and study discontinuation must be recorded in the appropriate section of the electronic case report form (eCRF).

8.3.1. Withdrawal From Study Drug

Subjects may be permanently withdrawn from study drug based on the judgment of the Investigator. Subjects who are withdrawn from study drug will be encouraged to return for the Follow-Up Visit, unless consent has been withdrawn. Subjects may be withdrawn from study drug under the following conditions:

- Withdrawal by subject
- Development of an intercurrent medical condition that precludes further participation in the study
- Development of an AE that meets the criteria for discontinuation from study drug as depicted in Section 9.3
- Subject requires use of a prohibited concomitant medication (Section 9.5.1)
- Persistent nonadherence to protocol requirements
- Lost to follow-up
- Approved drug available for indication
- Study termination
- Investigator decision
- Other

The Investigator should routinely assess the benefit/risk of continuing AG-348 to determine whether staying on the study drug is in the best interest of the subject. If questions arise, the Investigator may contact the Medical Monitor or designee.

Refer to Section 9.3 for details on discontinuing study drug.

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

8.3.2. Withdrawal From the Study

Subjects will be withdrawn from the study under the following conditions:

- Withdrawal of consent
- Development of an intercurrent medical condition that precludes further participation in the study
- Development of an AE that meets the criteria for discontinuation from study drug as depicted in Section 9.3
- Subject requires use of a prohibited concomitant medication (Section 9.5.1)
- Persistent nonadherence to protocol requirements
- Lost to follow-up
- Approved drug available for indication
- Study termination
- Investigator decision
- Other

8.3.3. Temporary Withdrawal From Study Drug for Pregnancy

Should a subject become pregnant during the study, the pregnancy should be reported to the Sponsor as detailed in Section 11.4, and the subject should interrupt treatment with AG-348 immediately and remain off study drug for the duration of pregnancy and breastfeeding. Because abrupt interruption of AG-348 dosing may result in withdrawal hemolysis, subjects should be monitored closely for signs of hemolysis and worsening or recurrence of anemia.

Pregnancy will not be considered a reason for subjects' permanent withdrawal from the study drug. After discussion with the Medical Monitor, or designee, the pregnant subject may be offered the opportunity to remain on the study with study drug interrupted while pregnant or breastfeeding. If the subject remains on the study, the subject will be expected to continue with regular visits. Pregnant subjects will not undergo DXA scans. CCI [redacted]. For subjects in Cohort 1 who become pregnant during the first 24 weeks of study participation, pharmacokinetic and PD assessments will not be performed at any visit after the last dose of AG-348 has been administered. Pharmacokinetic and PD assessments may be resumed if the subject in Cohort 1 eventually resumes study drug.

After completion of pregnancy and/or breastfeeding, the subject may be able to resume study drug; discussion with and approval by the Medical Monitor, or designee, will be required for re-initiation of study drug and to determine the timing and requirements for the start of study drug and assessments to be performed.

9. STUDY TREATMENTS

9.1. Description of Study Drug

Details on the study drug are provided in Table 1.

Table 1: Study AG348-C-011 Investigational Product

	Investigational product
Product name:	AG-348
Dosage form:	Tablets
Unit dose:	5 mg, 20 mg, and 50 mg
Route of administration:	Oral
Physical description:	Blue film-coated tablet

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.

9.1.1. Study Drug Packaging and Labeling

AG-348 will be supplied as 5, 20, or 50 mg tablets in 35-tablet bottles 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.

9.1.2. Study Drug Storage

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

9.1.3. 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 AG-348 may be taken with or without food. Subjects will take 1 tablet twice a day, approximately 12 hours (ie, 12 hours ± 2 hours) apart, for a total of 2 tablets each day (unless a dose modification is required for reasons related to safety).

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

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

discontinue study drug at any time during the study, guidance is provided in Section 9.3 and Section 7.2.5.

9.1.4. Study Drug Accountability

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

The Investigator or delegate will maintain accurate drug accountability records indicating the drug's delivery to the site and to the subject, inventory at the site, use by each subject, and return to the Sponsor or 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 during scheduled on-site study visits; under exceptional circumstances and with agreement of the Sponsor (or representative), study drug that was intended to be dispensed during scheduled on-site study visits can be provided at the subject's home, if acceptable by practice and allowed by local regulations. For scheduled telemedicine study visits (See Table 6), study drug will be shipped by the site to the subject's home if acceptable by practice and allowed by local regulations.

9.1.5. 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. Assignment of Subjects to Treatment

This is an open-label study, in which all subjects will be treated with AG-348.

CCI [redacted]

CCI [redacted]

9.3.2. Occurrence of Study Drug–Related Adverse Events CCI [redacted]

Dose modification that may be required because of study drug–related AEs CCI [redacted] are described in Table 2.

Table 2: Dose Modification for Adverse Events Considered Related to Study Drug CCI [redacted], Study AG348-C-011

Related AE(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 treatment abruptly or reducing the dose, the Investigator should determine which of the following options is appropriate: • Maintaining the current dose, **or** • Performing the recommended dose taper, **or** • Stopping the study 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

Table 2: Dose Modification for Adverse Events Considered Related to Study Drug CCI [redacted], Study AG348-C-011

Related AE(s) severity	Dose modification
	the event resolves to baseline[1] or Grade 1 (whichever is lower). If the event persists, performing the recommended dose taper or stopping the study drug abruptly should be considered. If the decision is made to perform the recommended dose taper or stop the study drug abruptly, the below instructions should be followed for re-introduction or re-escalation of the study drug, respectively. **In all cases, re-introduction and re-escalation of study drug should be performed only after discussion with the Medical Monitor or designee.** • Restarting study drug after dosing was stopped: – Once the event resolves to baseline[1] or Grade 1 (whichever is lower) and the decision is made to restart treatment, the study drug should be re-introduced at the 5 mg BID dose level. If the event does not re-occur after at least 4 weeks on 5 mg BID (with at least once weekly monitoring), the dose may be increased from 5 mg BID to 20 mg BID. If the event does not re-occur after at least 4 weeks on 20 mg BID (with at least once weekly monitoring), the dose may be increased from 20 mg BID to 50 mg BID. In both cases, dose escalation should follow the guidance in Section 7.2.2. • Events resolving during the recommended dose taper (ie, the subject is still on study drug): – If during the dose taper, the event resolves to baseline[1] 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 re-occur after at least 4 weeks, then consider increasing 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 re-occur after at least 4 weeks (with at least once weekly monitoring), and the subject is not already receiving 50 mg BID, consider increasing the dose to the next BID dose level. In both cases, dose escalation should follow the guidance in Section 7.2.2. • Re-occurrence of the AE: – If the AE re-occurs at any point during the above scenarios, the subject should undergo the recommended dose taper or stop treatment abruptly, if necessitated by the risk of the AE. If the subject undergoes the recommended dose taper and the AE resolves during the taper, study 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[1] or Grade 1 (whichever is lower) after the dose is decreased, a further decrease in the dose should be

Table 2: Dose Modification for Adverse Events Considered Related to Study Drug [redacted], Study AG348-C-011

Related AE(s) severity	Dose modification
	considered. If the AE still does not resolve, study drug should be permanently discontinued.
Grade 4	After careful consideration of the relative risk of withdrawal hemolysis when stopping study drug abruptly versus reducing the dose, the Investigator should determine which of the following options is appropriate: • Performing the recommended dose taper **or** • Stopping the study drug abruptly If the event resolves, and the Investigator believes that re-introducing study drug is justified, the Medical Monitor, or designee, should be consulted before any further study drug is administered.

Abbreviations: BID=twice daily; Hb=hemoglobin.
[1] In the context of dose modification due to an AE, baseline is defined as the baseline of the study (antecedent or extension) during which AE onset occurred. If an AE worsens, baseline refers to the study during the initial onset of the AE.

9.3.3. Study Drug Discontinuation or Interruption With Recommended Dose Taper Regimen

All subjects who discontinue or interrupt study drug during the study should undergo the recommended dose taper regimen in Table 3. This regimen is based on the study drug dose administered to the subject at the start of the taper and occurs in 1 or 2 sequential steps.

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

Table 3: Recommended Dose Taper Regimen, Study AG348-C-011

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

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

9.4. Duration of Subject Participation

9.4.1. Treatment Duration

Subjects may continue AG-348 treatment for up to a maximum of 192 weeks in this extension study (not including the time required for completion of the recommended dose taper) until they meet study withdrawal criteria (Section 8.3) or the study is closed (Section 7.5).

9.4.2. End of Study

The End of Study is defined as the point at which all subjects have discontinued or completed the study, are lost to follow-up, or the Study Sponsor terminates the study.

9.4.3. Treatment Compliance

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

9.5. Concomitant Medications

Concomitant medications are defined as those administered from the point of signing the informed consent form (ICF) on this extension study through the subject's completion of the study. Prior medications will have already been collected as either a prior medication or concomitant medication in the respective antecedent study and, thus, will not be collected at the start of this extension study. All 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 refer to Section 9.5.4.

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 (except for AG-348) no less than 3 months before the start of study drug on this extension study.

In vitro studies using human liver microsomes and recombinant CYP enzymes have shown that AG-348 is primarily metabolized by CYP3A4 and CYP3A5, with minor contributions from CYP2C9, CYP2C8, and CYP1A2. In addition, AG-348 has been shown to be a weak time-dependent CYP3A4/5 inhibitor and a potential inducer of CYP3A4 in vitro.

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)

  Note: If a subject is taking any medication that is a strong inhibitor of CYP3A4 before 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) before the start of study drug on this extension study.

- Products known to inhibit CYP3A4, such as grapefruit or grapefruit juice
- Strong inducers of CYP3A4 (listed in Appendix 5 of the AG-348 IB)

  Note: If a subject is taking any medication that is a strong inducer of CYP3A4 before enrolling in the study, the medication must be discontinued at least 28 days or a time frame equivalent to 5 half-lives (whichever is longer) before the start of study drug on this extension study.
- Hematopoietic stimulating agents (eg, erythropoietins, granulocyte colony stimulating factors, thrombopoietins) must be discontinued no less than 28 days before the start of study drug on this extension study. Vitamin B12 injections are permitted for subjects with a prior diagnosis of vitamin B12 deficiency syndromes. Subjects must be repleted to stability of the Hb and mean corpuscular volume before enrollment in this extension study.
- Anabolic steroids, including testosterone preparations, administered for anemia must be discontinued no less than 28 days before the start of study drug on this extension study.

9.5.2. Concomitant Therapy Requiring Careful Monitoring

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

- Corticosteroids (sensitive substrates of CYP3A4 and weak CYP3A4 inducers)
- Sensitive substrates of CYP3A4 (listed in Appendix 5 of the AG-348 IB)
- Moderate inhibitors of CYP3A4 (listed in Appendix 5 of the AG-348 IB)
- Moderate inducers of CYP3A4 (listed in Appendix 5 of the AG-348 IB)
- Proton-pump inhibitors and H2-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.
- Deferoxamine, deferasirox, and deferiprone. AG-348, as a potential uridine 5′-diphospho-glucuronosyltransferase (UGT)1A1 inducer, has the potential to reduce the effectiveness of iron chelators metabolized by UGT1A1. As iron overload is a long-term complication of PK deficiency, any initiation, completion, or change of iron chelation therapy during this extension study should be recorded in the appropriate section of the source documentation and the eCRF. Information to be recorded includes type of chelation therapy, start date, stop date, and dose.

AG-348, being a potential CYP3A4 inducer, has the potential to reduce the effectiveness of oral contraceptives. Therefore, women using oral contraceptives must also utilize a barrier method while enrolled in the study and until at least 28 days after their last dose of study drug, as specified in Inclusion Criterion #5 (Section 8.1).

9.5.3. 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, anti-emetics, anti-infectives, and antipyretics as medically indicated and consistent with the guidance in Section 9.5.1.

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

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

9.5.4. Concomitant Non-Drug Therapies

Concomitant non-drug therapies are defined as those performed from the point of signing the ICF on this extension study through the subject's completion of the study. As this is an extension study, prior non-drug therapies relevant for medical/surgical history will have been recorded with the antecedent study. Relevant concomitant non-drug therapies used to treat an AE should be collected from signing of ICF on this extension study until completion of the study.

For each transfusion administered at any time during the study, the date of transfusion and number of RBC units transfused will be recorded from signing of ICF until completion of the study.

9.6. Randomization and Blinding

Not applicable, as this is an open-label study in which all subjects will be treated with AG-348.

10. ASSESSMENTS

10.1. Schedule of Assessments

The schedules of assessments for Cohort 1 (through Week 24), Cohorts 2 and 3 (through Week 24), and Cohorts 1, 2, and 3 (Week 37 Day 1 through Week 193 Day 1) are provided in Table 4, Table 5, and Table 6, respectively.

Subjects may transition to receiving treatment with mitapivat (the international nonproprietary name of AG-348) outside of Study AG348-C-011 (eg, commercial) and may follow an altered schedule of assessments depending on whether they complete the study or prematurely discontinue, as described below:

- Subjects who complete study (ie, complete the last scheduled visit) before transitioning:
  - Subjects who intend to complete the study and immediately transition to receiving treatment with mitapivat outside of Study AG348-C-011 without interruption of mitapivat dosing may have their End of Study visit at Week 193. These subjects would not be dispensed study drug on Week 193, not be required to undergo the final dose taper, and not attend the Safety Follow-up visit.
- Subjects who prematurely discontinue study before transitioning:
  - Subjects who transition to receiving treatment with mitapivat outside of Study AG348-C-011 before their Week 193 visit will attend an End of Study visit that includes the same assessments that would have been performed on Week 193 and will be withdrawn from the study. These subjects will not be dispensed study drug at their End of Study visit and will not attend the Safety Follow-up visit. Subjects who intend to immediately transition to receiving treatment with mitapivat outside of Study AG348-C-011 without interruption of mitapivat dosing are not required to undergo the final dose taper.

As summarized in Section 9.3, withdrawal hemolysis is an identified risk of mitapivat, and subjects should undergo dose taper if treatment with mitapivat is to be discontinued or interrupted. For subjects who transition to receive treatment with mitapivat outside of Study AG348-C-011, please refer to the dose management instructions (eg, package insert) to assess the need for subjects to undergo dose taper during the transition.

Table 4: Schedule of Assessments for Cohort 1 Only (Day 1-Week 24)

Visit:	SCRN/D1[1]	W2	W4	W6	W8	W10[2]	W12	W16	W20	W24[3]	Safety Follow-up[4]
Study Day:	**1**	**15**	**29**	**43**	**57**	**71**	**85**	**113**	**141**	**169**	**28 days after the last dose of AG-348**
Visit Window:	**0**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±4 D**
Procedures:											
Informed consent	X										
Eligibility	X										
Demographics[5]	X										
Physical examination[6]	X						X			X	
Vital signs[7]	X	X	X	X	X		X	X	X	X	X
12-lead ECG[8]	X						X			X	X
DXA Scan	X									X	
CCI [redacted]	[redacted]									[redacted]	
AEs/SAEs/AESIs[9]	X										
Concomitant medications/non-drug therapies	X										
On-study transfusion record[10]	X										
Clinical Laboratory Evaluations:											
Hematology[11]	X	X	X	X	X	X	X	X	X	X	X
Haptoglobin and LDH	X	X	X	X	X	X	X	X	X	X	X
Serum chemistry[12]	X						X			X	X
LFTs[13]	X	X	X	X	X	X	X	X	X	X	X
Coagulation studies[14]	X						X			X	
Urinalysis[15]	X						X			X	
Lipids[16]	X		X		X		X	X	X	X	X

Table 4: Schedule of Assessments for Cohort 1 Only (Day 1-Week 24)

Visit:	SCRN/D1[1]	W2	W4	W6	W8	W10[2]	W12	W16	W20	W24[3]	Safety Follow-up[4]
Study Day:	**1**	**15**	**29**	**43**	**57**	**71**	**85**	**113**	**141**	**169**	**28 days after the last dose of AG-348**
Visit Window:	**0**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±3 D**	**±4 D**
Sex Steroids[17]	X		X		X		X	X	X	X	X
CCI [redacted] CRP	X						X			X	
EPO CCI [redacted]	X						X			X	
T4, TSH, PTH, fructosamine, and Vitamin D	X						X			X	
FSH[20]	X										
Pregnancy test (local laboratory)[21]	X		X		X		X	X	X	X	X
HRQoL Assessments and Other eDiary Assessments:[22]											
PKDD[23]	X-Daily-X										
PKDIA			X		X		X	X	X	X	
Dispense eDiary	X										
Menstrual cycle eDiary	X										
Return eDiary											X
Pharmacokinetic and Pharmacodynamic Assessments:											
Pharmacokinetic full-profile sampling[24]							X				
Pharmacokinetic sparse blood sampling[25]	X	X		X				X			
CCI [redacted]							[redacted]				
Study Drug:											
Dispense study drug[27]	X		X		X		X	X	X	X	
Study drug administration[28]	X										
Return study drug			X		X		X	X	X	X	X

Abbreviations: AE = adverse event; AESI = adverse event of special interest; ALP = alkaline phosphatase; ALT = alanine aminotransferase; aPTT = activated partial thromboplastin time; AST = aspartate aminotransferase; BUN = blood urea nitrogen; CBC = complete blood count; CO_2 = carbon dioxide; CRP = C-reactive protein; D = day; DXA = dual-energy X-ray absorptiometry; ECG = electrocardiogram; eDiary = electronic diary; EPO = erythropoietin; FSH = follicle-stimulating hormone; Hb = hemoglobin; HCT = hematocrit; HDL-C = high-density lipoprotein cholesterol; HRQoL = health-related quality of life; ICF = informed consent form; INR = international normalized ratio; IRF = immature reticulocyte fraction; LDH = lactate dehydrogenase; LDLC = low-density lipoprotein cholesterol; LFT = liver function test; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; CCI NRBC = nucleated red blood cells; CCI ; PE = physical examination; PKDD = Pyruvate Kinase Deficiency Diary; PKDIA = Pyruvate Kinase Deficiency Impact Assessment; CCI ; PTH = parathyroid hormone; RBC = red blood cell; RDW = red cell distribution width; SAE = serious adverse event; SCRN = Screening; T4 = thyroxine; CCI T_{max} = time to maximum (peak) concentration; TSH = thyroid-stimulating hormone; W = week; WBC = white blood cell.

Whenever more than one assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (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 the Medical Monitor, or designee, and the site.

[1] A subject's first visit of this extension study should coincide with his/her last visit of Study AG348-C-006. Assessments that overlap between the last visit of Study AG348-C-006 and screening of this extension study only need to be performed once. All assessments for eligibility for this extension study must be completed before a subject can begin study drug in this extension study. The first visit of this extension study should consist of subject consent, screening, confirmation of eligibility, and the start of study drug. If a subject in Cohort 1 cannot start study drug on the first visit of this extension study, discussion with and approval by the Medical Monitor, or designee, will be required for participation in this extension study, and to determine the timing and requirements for the start of study drug and assessments to be performed.

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

[3] If a subject in Cohort 1 is continuing in the study beyond the Week 24 Visit, refer to the schedule of assessments in Table 6 for all assessments beyond Week 24.

[4] A Safety Follow-up Visit will be performed for all subjects who permanently discontinue study drug. The visit will occur 28±4 days after the last dose of study drug (including the time required to dose taper).

[5] Subject demographic data will include gender and year of birth only, as other demographic data will have been collected as part of the antecedent study.

[6] Complete PE with weight will be collected. Additional PEs may be performed when clinically indicated, at the discretion of the Investigator.

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

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

[9] AEs/SAEs/AESIs will be collected and recorded as detailed in Section 11.1. The Investigator should ask the subject for information regarding sleep patterns, signs, and symptoms associated with insomnia.

[10] Transfusion records will be kept while subjects are on study and will include the date of the transfusion and the number of RBC units transfused.

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

[12] Serum chemistry parameters include sodium, potassium, chloride, calcium, magnesium, phosphorus, CO_2 or bicarbonate, albumin, total protein, glucose, BUN or urea, creatinine, and uric acid.

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

[14] Coagulation parameters include fibrinogen, aPTT, and INR.

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

[16] For lipid testing, samples for total cholesterol, LDL-C, HDL-C, and triglycerides 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 includes estrone, estradiol, and testosterone (total and free).

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[19] Remaining sample may be used for analysis of lipoproteins (only in subjects who have agreed to this optional analysis in the ICF).

[20] 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), unless an elevated FSH level indicative of menopause was obtained during the antecedent study. Samples should be drawn in the morning (does not need to be fasting).

[21] For women of reproductive potential in Cohort 1, a local serum pregnancy test must be completed during screening; if the result of the local serum test will not be available on the same day, a local urine pregnancy test must also be completed. Results from the local serum test or the local urine test (if the serum test results are not available) must be documented to be negative before the subject can start study drug in this extension study. If a subject in Cohort 1 does not start study drug on the same day as screening, a local urine pregnancy test must be documented as negative prior to starting study drug. A local urine or serum pregnancy test must also be done every 4 weeks and at any point throughout the study if pregnancy is clinically suspected.

[22] All subjects will use an eDiary to record responses to HRQoL assessments. The HRQoL assessments will be completed by the subject in the evening (between 5:00-11:00 pm) within the window for the relevant study visit. 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. Subjects should return their eDiary to the study site once they complete the study at the Safety Follow-up Visit.

[23] The PKDD assessment will be collected daily from screening through the Week 24 Visit.

[24] Pharmacokinetic full profile blood sampling will be conducted in all subjects at the Week 12 Visit at the following time points: predose (within 60 minutes prior to study drug administration) and 30 minutes (±5 minutes), 1 hour (±5 minutes), 2 hours (±5 minutes), 4 hours (±30 minutes), and 8 hours (±30 minutes) post study drug administration. At this visit, the morning dose of study drug must be administered at the study site; since a predose sample is required at this visit, the study drug must be administered after the predose sample is taken.

[25] Pharmacokinetic sparse blood sampling collection times are as follows: First day of study drug (predose; within 60 minutes prior to study drug administration), Week 2 (1-2 hours and 3-4 hours post-study drug administration), Week 6 (4-5 hours and 6-7 hours post-study drug administration), and Week 16 (predose; within 60 minutes prior to study drug administration). On days where a pre-dose blood sample collection is required, the study drug must be administered after the pre-dose sample is taken. On the first day of study drug, after the pre-dose sample is collected, the study drug must be administered at the study site. Starting from Week 2, on visits when pharmacokinetic blood sample collection is required, the morning dose of study drug must be administered at the study site.

C[redacted]

C[redacted]

[27] Study drug is expected to be dispensed to the subject at the study site. Under exceptional circumstances and with agreement of the Sponsor (or representative), study drug can be provided at the subject's home (if acceptable by practice and allowed by local regulations). All subjects who discontinue or interrupt AG-348 at any time should undergo the recommended dose taper per protocol (Section 9.3), unless an emergency situation justifies discontinuing or interrupting the study drug abruptly. Whether the dose taper is performed or not, subjects discontinuing or interrupting AG-348 should be monitored for signs of hemolysis and worsening of anemia. Study drug will be dispensed to these subjects until study drug is completely stopped.

[28] For Cohort 1, the first dose of AG-348 in this extension study should be administered at the study site.

Table 5: Schedule of Assessments for Cohorts 2 and 3 Only (Day 1-Week 24)

Visit:	SCRN/D1[1]	W12	W24[2]	Safety Follow-up[3]
Study Day:	**1**	**85**	**169**	**28 days after the last dose of AG-348**
Visit Window:	**0**	**±3 D**	**±3 D**	**±4 D**
Procedures:				
Informed consent	X			
Eligibility	X			
Demographics[4]	X			
Physical examination[5]	X	X	X	
Vital signs[6]	X	X	X	X
12-lead ECG[7]	X	X	X	X
DXA Scan	X		X	
CCI	[redacted]		[redacted]	
AEs/SAEs/AESIs[8]	X			
Concomitant medications/non-drug therapies	X			
On-study transfusion records[9]	X			
Clinical Laboratory Evaluations:				
Hematology[10]	X	X	X	X
Haptoglobin and LDH	X	X	X	X
Serum chemistry[11]	X	X	X	X
LFTs[12]	X	X	X	X
Coagulation studies[13]	X	X	X	
Urinalysis[14]	X	X	X	
Lipids[15]	X	X	X	X
Sex Steroids[16]	X	X	X	X

Table 5: Schedule of Assessments for Cohorts 2 and 3 Only (Day 1-Week 24)

Visit:	SCRN/D1[1]	W12	W24[2]	Safety Follow-up[3]
Study Day:	**1**	**85**	**169**	**28 days after the last dose of AG-348**
Visit Window:	**0**	**±3 D**	**±3 D**	**±4 D**
CCI CRP	X	X	X	
EPO CCI	X	X	X	
T4, TSH, PTH, fructosamine, and Vitamin D	X	X	X	
FSH[19]	X			
Pregnancy test (local laboratory and home testing) [20]	X	X	X	X
HRQoL Assessments and Other eDiary Assessments:[21]				
PKDD[22]		X	X	
PKDIA		X	X	
Dispense eDiary	X			
Menstrual cycle eDiary	X			
Return eDiary				X
Study Drug:				
Dispense study drug[23]	X	X	X	
Study drug administration	X			
Return study drug		X	X	X

Abbreviations: aPTT = activated partial thromboplastin time; BUN = blood urea nitrogen; CBC = complete blood count; CO_2 = carbon dioxide; CRP = C-reactive protein; D = day; DXA = dual-energy X-ray absorptiometry; ECG = electrocardiogram; eDiary = electronic diary; EPO = erythropoietin; FSH = follicle-stimulating hormone; Hb = hemoglobin; HCT = hematocrit; HDL-C = high-density lipoprotein cholesterol; HRQoL = health-related quality of life; ICF = informed consent forum; INR = international normalized ratio; IRF = immature reticulocyte fraction; LDH = lactate dehydrogenase; LDLC = low-density lipoprotein cholesterol; LFT = liver function test; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; CCI NRBC = nucleated red blood cells; CCI PE = physical examination; PKDD = Pyruvate Kinase Deficiency Diary; PKDIA = Pyruvate Kinase Deficiency Impact Assessment; PTH = parathyroid hormone; RBC = red blood cell; RDW = red cell distribution width; SCRN = Screening; T4 = thyroxine; CCI TSH = thyroid-stimulating hormone; W = week; WBC = white blood cell.

Whenever more than one assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (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 the Sponsor's Medical Monitor, or designee, and the site.

[1] A subject's first visit of this extension study should coincide with his/her last visit of their antecedent study (ie, Study AG348-C-006 or Study AG348-C-007). Assessments that overlap between the last visit of the antecedent study and screening of this extension study only need to be performed once. All assessments for eligibility for this extension study must be completed before a subject can start study drug in this extension study. The first visit of this extension study for subjects on Cohorts 2 and 3 will consist of screening, confirmation of eligibility, and the start of study drug on this extension study.
[2] If a subject in Cohort 2 or 3 is continuing in the study beyond the Week 24 Visit, refer to the schedule of assessments in Table 6 for all assessments beyond Week 24.
[3] A Safety Follow-up Visit will be performed for all subjects who permanently discontinue study drug. The visit will occur 28±4 days after the last dose of study drug (including the time required to dose taper).
[4] Subject demographic data will include gender and year of birth only, as other demographic data will have been collected as part of the antecedent study.
[5] Complete PE with weight will be collected. Additional PEs may be performed when clinically indicated, at the discretion of the Investigator.
[6] Vital signs will include systolic and diastolic blood pressure, heart rate, and body temperature.
[7] The 12-lead ECGs will be conducted using the equipment provided by the vendor and according to the vendor manual, after 5 minutes of recumbence and in triplicate. The ECGs will be read promptly by a qualified physician at the study site to detect any safety issue. In addition to the local read, the ECGs will be sent promptly to the central vendor for a data-analysis read.
[8] AEs/SAEs/AESIs will be collected and recorded as detailed in Section 11.1. The Investigator should ask the subject for information regarding sleep patterns, signs, and symptoms associated with insomnia.
[9] Transfusion records will be kept while subjects are on study and will include the date of the transfusion and the number of RBC units transfused.
[10] Hematology parameters (ie, CBC with differential) include HCT, Hb, RBC count, percent reticulocyte, absolute reticulocyte count, immature reticulocyte fraction (IRF-H and IRF-M+H) (if available), MCV, MCH, MCHC, RDW, NRBC, WBC count with differential, and platelet count.
[11] Serum chemistry parameters include sodium, potassium, chloride, calcium, magnesium, phosphorus, CO_2 or bicarbonate, albumin, total protein, glucose, BUN or urea, creatinine, and uric acid.
[12] Liver function tests include ALP, ALT, AST, and total, direct, and indirect bilirubin.
[13] Coagulation parameters include fibrinogen, aPTT, and INR.
[14] Urinalysis will be performed by a dipstick method and include assessments of protein, glucose, leukocytes, and blood.
[15] For lipid testing, samples for total cholesterol, LDL-C, HDL-C, and triglycerides 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 an unscheduled visit, after the subject has adhered to an overnight fast.
[16] Sex steroid testing includes estrone, estradiol, and testosterone (total and free).
[17] [redacted]
[18] Remaining sample may be used for analysis of lipoproteins (only in subjects who have agreed to this optional analysis in the ICF).
[19] 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), unless an elevated FSH level indicative of menopause was obtained at screening of the antecedent study. Samples should be drawn in the morning (does not need to be fasting).
[20] For women of reproductive potential on Cohorts 2 and 3, a local urine pregnancy test must be completed during screening and documented to be negative before the subject can start study drug in this extension study. A urine or serum pregnancy test must also be done every 4 weeks and at any point throughout the study if pregnancy is clinically suspected. A local urine or serum pregnancy test should be performed at the study site at the scheduled visits and a urine pregnancy test should be performed by the subject at home when required testing occurs between the scheduled visits. Results of the home pregnancy tests will be recorded in the eDiary.
[21] All subjects will use an eDiary to record responses to HRQoL assessments and results of home pregnancy tests (women of reproductive potential only). The HRQoL assessments will be completed by the subject in the evening (between 5:00-11:00 pm) within the window for the relevant study visit. 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. Subjects should return their eDiary to the study site once they complete the study at the Safety Follow-up Visit.
[22] The PKDD assessment will be collected for 6 days prior to and on the day of the PKDIA assessment.
[23] Study drug is expected to be dispensed to the subject at the study site. Under exceptional circumstances and with agreement of the Sponsor (or representative), study drug can be provided at the subject's home (if acceptable by practice and allowed by local regulations). All subjects who discontinue or interrupt AG-348 at any time should undergo the recommended dose taper per protocol (Section 9.3), unless an emergency situation justifies discontinuing or interrupting the study drug abruptly. Whether the dose taper is

performed or not, subjects discontinuing or interrupting AG-348 should be monitored for signs of hemolysis and worsening of anemia. Study drug will be dispensed to these subjects until study drug is completely stopped.

Table 6: Schedule of Assessments for All Cohorts, Continued Treatment Period (W37D1-W193D1)

Visit:	W37D1 W61D1 W85D1	W49D1 W97D1	W73D1	W109D1 W133D1 W157D1 W181D1 (Telemedicine[1])	W121D1 W169D1	W145D1	W193D1[2]	Safety Follow-up[2,3] 28 days after the last dose of AG-348
Visit window:	**±14 D**	**±14 D**	**±14 D**	**±14 D**	**±14 D**	**±14 D**		**±4 D**
Procedures:								
Physical examination[4]							X	
Vital signs[5]	X	X	X		X	X	X	X
DXA scan[2]		X	X			X	X	
CCI [redacted]		[redacted]	[redacted]			[redacted]	[redacted]	
Reproductive potential[6]	X							
AEs/SAEs[7]	X							
Concomitant medications/non-drug therapies	X							
Record on-study transfusions[8]	X							
Clinical laboratory evaluations:								
Hematology[9]	X	X	X		X	X	X	X
Haptoglobin and LDH	X	X	X		X	X	X	X
Serum chemistry[10]		X	X		X	X	X	X
LFTs[11]	X	X	X		X	X	X	X
Coagulation studies[12]		X	X			X	X	
Lipids[13]			X		X			X
Sex Steroids[14]	X	X	X		X	X	X	X
[redacted] CCI [redacted] [redacted] CRP	X	X	X		X	X	X	

Table 6: Schedule of Assessments for All Cohorts, Continued Treatment Period (W37D1-W193D1)

Visit:	W37D1 W61D1 W85D1	W49D1 W97D1	W73D1	W109D1 W133D1 W157D1 W181D1 (Telemedicine[1])	W121D1 W169D1	W145D1	W193D1[2]	Safety Follow-up[2,3] 28 days after the last dose of AG-348
Visit window:	**±14 D**	**±14 D**	**±14 D**	**±14 D**	**±14 D**	**±14 D**		**±4 D**
EP [redacted]	X	X	X		X	X	X	
T4, TSH, PTH, fructosamine, and vitamin D		X	X		X	X	X	
Study drug:								
Dispense study drug[17]	X	X	X	X	X	X	X[2]	
Study drug administration	X							
Return study drug	X	X	X		X	X	X	X

Abbreviations: aPTT = activated partial thromboplastin time; BUN = blood urea nitrogen; CBC = complete blood count; CO2 = carbon dioxide; CRP = C-reactive protein; D = day; DXA = dual-energy X-ray absorptiometry; eDiary = electronic diary; EPO = erythropoietin; Hb = hemoglobin; HCT = hematocrit; HDL-C = high-density lipoprotein cholesterol; ICF = informed consent form; INR = international normalized ratio; IRF = immature reticulocyte fraction; LDH = lactate dehydrogenase; LDL-C = low-density lipoprotein cholesterol; LFT = liver function test; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; [redacted] NRBC = nucleated red blood cells; PE = physical examination; PKDD = Pyruvate Kinase Deficiency diary; PKDIA = Pyruvate Kinase Deficiency Impact Assessment; PKR = red blood cell-specific form of pyruvate kinase; PTH = parathyroid hormone; RBC = red blood cell; RDW = red cell distribution width; T4 = thyroxine; [redacted] TSH = thyroid-stimulating hormone; W = week; WBC = white blood cell.

Whenever more than one assessment is scheduled for the same nominal time, the assessments should be performed in the order of least invasive to most invasive assessment (eg, vital signs, 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 the Sponsor's Medical Monitor, or designee, and the site.

[1] Telemedicine visits will be conducted via phone or video call to assess current supply of study drug, assess changes in underlying health status, and record AEs, concomitant medications, and transfusions. Investigators should use prudent medical judgment to determine whether to request for subjects to attend unscheduled on-site visits based on information gathered at a telemedicine visit. For sites where telemedicine is not permitted by local regulations, subjects are to complete their assessments in-person at the site.

[2] Subjects who intend to complete the study and immediately transition to receiving treatment with mitapivat outside of Study AG348-C-011 without interruption of mitapivat dosing may have their End of Study visit at Week 193. Subjects who transition to receiving treatment with mitapivat outside of Study AG348-C-011 before their Week 193 Visit will attend an End of Study visit that includes the same assessments that would have been performed on Week 193 and will be withdrawn from the study. These subjects would not be dispensed study drug at the End of Study visit and not attend the Safety Follow-up Visit. Subjects who intend to immediately transition to receiving treatment with mitapivat outside of Study AG348-C-011 without interruption of mitapivat dosing are not required to undergo the final dose taper. [redacted] DXA scans are not required to be performed at the End of Study visit if those assessments were performed within the past 6 months.

[3] A Safety Follow-up Visit will be performed for all subjects who permanently discontinue study drug. The visit will occur 28±4 days after the last dose of study drug (including the time required to dose taper).

[4] Complete PE with weight will be collected. Additional PEs may be performed when clinically indicated, at the discretion of the Investigator.
[5] Vital signs will include systolic and diastolic blood pressure, heart rate, and body temperature.
[6] Changes in reproductive potential of female subjects (as defined in Appendix 1) will be recorded, including the date of the change.
[7] AEs/SAEs will be collected and recorded as detailed in Section 11.1. The Investigator should ask the subject for information regarding sleep patterns, signs, and symptoms associated with insomnia. CCI [redacted]
[8] Transfusion records will be kept while subjects are on study and will include the date of the transfusion and the number of RBC units transfused.
[9] Hematology parameters (ie, CBC with differential) include HCT, Hb, RBC count, percent reticulocyte, absolute reticulocyte count, immature reticulocyte fraction (IRF-H and IRF-M+H) (if available), MCV, MCH, MCHC, RDW, NRBC, WBC count with differential, and platelet count.
[10] Serum chemistry parameters include sodium, potassium, chloride, calcium, magnesium, phosphorus, CO_2 or bicarbonate, albumin, total protein, glucose, BUN or urea, creatinine, and uric acid.
[11] Liver function tests include ALP, ALT, AST, and total, direct, and indirect bilirubin.
[12] Coagulation parameters include fibrinogen, aPTT, and INR.
[13] Lipid tests include total cholesterol, LDL-C, HDL-C, and triglycerides.
[14] Sex steroid testing includes estrone, estradiol, and testosterone (total and free).
C [redacted]
[16] Remaining sample may be used for analysis of lipoproteins (only in subjects who have agreed to this optional analysis in the ICF).
[17] Study drug is expected to be dispensed to the subject at the study site during scheduled on-site study visits; under exceptional circumstances and with agreement of the Sponsor (or representative), study drug that was intended to be dispensed during scheduled on-site study visits can be provided at the subject's home (if acceptable by practice and allowed by local regulations). For scheduled telemedicine study visits, study drug will be shipped by the site to the subject's home if acceptable by practice and allowed by local regulations. All subjects who discontinue or interrupt AG-348 at any time should undergo the recommended dose taper per protocol (Section 9.3), unless an emergency situation justifies discontinuing or interrupting the study drug abruptly. Whether the dose taper is performed or not, subjects discontinuing or interrupting AG-348 should be monitored for signs of hemolysis and worsening of anemia. Study drug will be dispensed to these subjects until study drug is completely stopped.

10.2. Screening Assessments

10.2.1. Informed Consent

Informed consent will be obtained after the completion of all assessments at the subject's last visit of his/her antecedent study and, for subjects in Cohorts 1 and 2, after the subject, Investigator, and site personnel are unblinded to the Study AG348-C-006 treatment allocation (ie, AG-348 or placebo), but before screening assessments not overlapping with the last visit of the antecedent study are performed and eligibility for this extension study is assessed by the Investigator.

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 or assessment of AG-348 metabolism; subjects may opt-in or decline; this will not affect the subjects' eligibility for the study.

10.2.2. Confirmation of Eligibility

A subject's eligibility will be assessed by the Investigator at screening. Assessments to be performed at screening are provided in the schedule of assessments for Cohort 1 in Table 4 and for Cohorts 2 and 3 in Table 5.

10.3. Medical History

Medical history will not be collected in this extension study, as this information will have already been collected as part of the antecedent study.

10.4. Demographics

Demographic data will include gender and year of birth only, as other demographic data will have been collected as part of the antecedent study.

10.5. Assessments of Safety

The timing of safety assessments is provided in the Schedule of Assessments (Section 10.1; Table 4, Table 5, and Table 6). Assessments of safety will include the following:

- Adverse event reporting, as discussed in Section 11.1
- Concomitant medications and non-drug therapies, as discussed in Section 9.5.3 and Section 9.5.4, respectively
- Physical examinations and vital sign collections
- Procedural assessments: ECG and DXA as detailed in Section 10.5.1 and Section 10.5.2, respectively
- Laboratory assessments as detailed in Section 10.5.3
- Menstrual cycle diary and reproductive potential as detailed in Section 10.5.4

10.5.1. Electrocardiogram

The 12-lead ECGs should be performed after 5 minutes of recumbence and in triplicate, using the ECG machine provided by the central vendor and according to the vendor manual. For Cohort 1 only, the ECG at Week 12 will be performed before, 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 ECGs will be read promptly by a qualified physician at the study site to detect any safety issue. 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.

As of Protocol Amendment 4, Version 4.0, ECGs are no longer required to be assessed after the Week 24 visit; however, ECGs may be performed at the discretion of the Investigator when clinically indicated.

10.5.2. 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 if not technically adequate. The DXA scans will be read and interpreted by the central vendor.

10.5.3. Safety Laboratory Assessments

All clinical laboratory evaluations are to be analyzed by a central laboratory, except for urine or serum pregnancy tests, which can be performed and analyzed by a local laboratory at scheduled visits or by home testing (urine) for tests required between scheduled visits. If results from a central laboratory are not available to support necessary clinical decision-making, then results from a local laboratory may be used. Blood samples for the Week 10 assessments in Cohort 1 may be collected outside of the study site and sent to the central laboratory by qualified personnel (eg, home health care nurse), if allowed by local regulations.

If Investigators believe that it is clinically indicated to obtain safety laboratory results from their own local laboratories on the day of the subject's visit, they are free to exercise their discretion to do so.

Blood samples for clinical laboratory evaluations will be collected according to the Schedule of Assessments (Section 10.1; Table 4, Table 5, and Table 6). All clinically significant laboratory abnormalities noted on testing will be followed by repeat testing and further investigation according to the judgment of the Investigator.

The following safety laboratory parameters will be measured:

- Complete blood count (CBC) with differential
- Serum chemistry
- LFTs
- Coagulation studies
- Sex steroids
- Lipids, measured by standard method and nuclear magnetic resonance
- Urinalysis
- Serum or urine pregnancy tests (for women of reproductive potential)
  - As of Protocol Amendment 4, Version 4.0, pregnancy tests are no longer required to be assessed after the Week 24 visit; however, pregnancy testing should occur more frequently if clinically indicated (ie, if there is suspicion of pregnancy) or required by local regulations.

10.5.4. Menstrual Cycle Diary and Assessments of Reproductive Potential

Menstruating female subjects will be required to fill out an electronic menstrual cycle diary according to the Schedule of Assessments (Section 10.1; Table 4 and Table 5). Subjects will record the start date, stop date, and change in characteristics of each menstrual cycle in the eDiary provided during screening. The menstrual cycle diary can be completed at any time of day.

After Week 24, female subjects will no longer fill out the menstrual cycle diary. Subjects should return the eDiary to the site at or before the End of Study visit.

Starting at Week 73, changes in reproductive potential of female subjects (as defined in Appendix 1) will be recorded in the source documents and eCRF, including the date of the change.

10.6. Assessments of Efficacy

The timing of efficacy assessments is provided in the Schedule of Assessments (Section 10.1; Table 4, Table 5, and Table 6).

All clinical laboratory evaluations for efficacy are to be performed by the central laboratory. If results from a central laboratory are not available to support necessary clinical decision-making, then results from a local laboratory may be used.

10.6.1. Hemoglobin Laboratory Assessment

Hemoglobin concentrations (as part of the CBC with differential) will be collected to support efficacy (and safety) assessments.

10.6.2. Transfusions

Transfusion data will be collected from signing of ICF for this extension study until completion of the study as noted in Section 9.5.4.

10.6.3. Additional Laboratory Assessments for Efficacy

The following laboratory parameters will be collected for additional efficacy assessments:

- RBC parameters, including reticulocyte counts and percentages
- Haptoglobin
- Bilirubin
- LDH
- EPO
- Erythroferrone
- Soluble transferrin receptor

10.7. Pharmacokinetic Assessments

10.7.1. Blood Sample Collection

Blood samples for a full pharmacokinetic profile will be collected from subjects in Cohort 1 at the Week 12 Visit at multiple time points as specified in the Schedule of Assessments (Section 10.1; Table 4).

Sparse blood samples for pharmacokinetics will be collected from subjects in Cohort 1 on the first day of study drug, Week 2, Week 6, and Week 16 visits. For details on the sparse sampling schedule, please refer to the Schedule of Assessments (Section 10.1; Table 4).

On days where a predose blood sample collection is required, the study drug must be administered after the predose sample is taken. On the first day of study drug, after the predose sample is collected, the study drug must be administered at the study site. Starting from Week 2, on visits when pharmacokinetic blood sample collection is required, the morning dose of study drug must be administered at the study site.

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.

10.7.2. 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
- $t_{1/2}$: Terminal half-life
- CL/F: The apparent total plasma clearance (CLp) after oral (extravascular) dosing
- Vz/F: Volume of distribution during the terminal elimination phase after oral (extravascular) dosing

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[redacted]

10.9. Health-Related Quality of Life Assessments

The timing of HRQoL assessments is provided in the Schedule of Assessments (Section 10.1; Table 4 and Table 5). The following HRQoL assessments will be performed:

- PKDD: 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.
- PKDIA: 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.

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10.10. Other Assessments

CCI [redacted]

10.10.2. Other Laboratory Assessments

All clinical laboratory evaluations are to be performed by a central laboratory. The following other laboratory parameters will be collected:

- CCI [redacted] related markers (remaining sample may be used for analysis of lipoproteins [only in subjects who have agreed to this optional analysis in the ICF])
- Assessments of complications of iron overload: thyroxine (T4), thyroid-stimulating hormone (TSH), parathormone (PTH), fructosamine, and vitamin D
- FSH (to confirm postmenopausal status)

11. ADVERSE EVENTS

11.1. Reporting Period for Adverse Events and Serious Adverse Events

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Monitoring of AEs, including frequency, severity, and characterization of SAEs, AESIs, and AEs leading to discontinuation will be conducted throughout the study. Beginning at the signing of the ICF of this extension study, all new, worsening, or ongoing AEs and SAEs will be recorded in the source documentation and eCRF for this extension study 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 (ie, baseline of the study during which AE onset occurred), the AE is considered stable within the context of the study, the subject is lost to follow-up, or until 28 days after the last dose of study drug.

All SAEs will be followed until final outcome of the SAE is known or the subject is lost to follow-up. Any SAEs that are assessed as related to the 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 as per Section 11.3. Any AEs already documented at a previous assessment and designated as ongoing will be reviewed at subsequent visits or assessment time points as necessary. If these AEs have resolved, this will be documented.

All AEs will be graded using the National Cancer Institute (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.

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 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 before study entry are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected manner during the study (eg, surgery performed earlier than planned).
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital anomaly/birth defect
- Important medical event. An important medical event is an event that may not result in death, be life-threatening, or require hospitalization, but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in the definitions for SAEs. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

11.2.3. Adverse Events Occurring Secondary to Other Events

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

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

11.2.4. Preexisting Medical Conditions

Preexisting medical conditions will not be collected in this extension study, as this information will have already been collected as part of the antecedent study.

11.2.5. Abnormal Laboratory Values

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

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

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

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

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

11.2.6. Adverse Events of Special Interest

CCI [redacted]

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.

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

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

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

All AEs (serious and nonserious) spontaneously reported by the subject and/or in response to an open question from study personnel or revealed by observation, PE, or other diagnostic procedures will be recorded on the appropriate page of the eCRF. Any clinically relevant deterioration in laboratory assessments or other clinical finding is considered an AE and must be recorded on the appropriate pages of the eCRF. When possible, signs and symptoms indicating a common underlying pathology should be noted as 1 comprehensive event.

Any deaths and any AEs assessed as life-threatening are to be reported immediately. All SAEs are to be reported within 24 hours from the point in time when the Investigator becomes aware of the SAE on the appropriate page of the eCRF. All SAEs must be reported whether or not they are considered causally related to the study 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 higher than the subject's ULN as applies to men and women). Any reports of excessive Hb response should be graded using the CTCAE grading system.

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

11.3.1. Intensity

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

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

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

11.3.2. Relationship to Study 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, preexisting 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 interruption 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 after the last dose of study drug must be reported to the Sponsor's Medical Monitor, or designee, within 24 hours of being notified of the pregnancy.

The Investigator must follow up and document the course and outcome of all pregnancies even if the subject was discontinued from the study or if the study has finished. The female 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 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 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 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 after the last dose of study drug. Periodic abstinence (eg, calendar, symptothermal, and postovulation methods) and withdrawal are not acceptable methods of contraception.

12. STATISTICAL METHODS

12.1. Analysis Sets

All subjects refers to all subjects enrolled in Study AG348-C-011.

- Safety Analysis Set (SAS) is defined as all subjects who are exposed to any amount of study drug in this extension study. The SAS will be used for both efficacy and safety analyses unless otherwise specified in the detailed Statistical Analysis Plan (SAP).

12.2. Statistical Analysis

12.2.1. General Methods

The analysis will only focus on data from this extension study (ie, not the antecedent studies). Details will be specified in the SAP.

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

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

Categorical variables will be summarized using counts and percentages.

Baseline value: The baseline value is defined as the most recent measurement before intake of the first dose of AG-348, except if specified otherwise. The first dose of AG-348 refers to the first dose in this extension study for Cohort 1, while it refers to the first dose in the antecedent study for Cohorts 2 and 3.

- The individual subject's baseline Hb and transaminase (AST and ALT) is defined as the average of all available Hb concentrations and transaminase (AST and ALT) values, respectively, collected for that subject within 42 days before the first dose of AG-348.

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

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

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

12.2.2. Background Characteristics

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

12.2.2.1. Subject Disposition

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

- All Subjects
- SAS

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

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

12.2.2.2. Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized and presented for the SAS.

12.2.2.3. Prior and Concomitant Medications and Procedures

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

- **Prior medication:** not collected in this trial
- **Concomitant medication/procedure:** medication/procedure continued or newly received from signing the ICF on this extension study to 28 days after the last dose of study drug
- **Posttreatment medication:** medication continued or newly received after the treatment-emergent period

A given medication can be classified as a concomitant medication, a posttreatment medication, or a concomitant and posttreatment medication. 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 a concomitant and posttreatment medication.

Concomitant medications will be summarized descriptively based on the SAS. Concomitant medications and posttreatment medications will all be listed for each subject.

Concomitant procedures will be listed.

12.2.3. Study Drug Exposure and Compliance

12.2.3.1. 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 drug discontinuation or completion date will be used for analysis purpose.

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

Exposure summaries will be based on the SAS.

12.2.3.2. Study Drug Compliance

Study drug compliance will be assessed by percentage of tablets taken.

The percent of tablets taken will be calculated as follows:

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

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

Study drug compliance will be based on the SAS.

12.3. Efficacy Analyses

12.3.1. Analysis for the Primary Endpoint

Not applicable, as the study's primary endpoint is safety (Section 12.4).

12.3.2. Analyses for the Secondary Endpoints

12.3.2.1. Hemoglobin-related Secondary Endpoints

The number and percentage of subjects experiencing an Hb response will be summarized for Cohort 1 subjects in the SAS based on the extension study assessments. If less than 2 out of the targeted 3 assessments are available, the subject will be considered a non-responder. Hemoglobin values assessed within 2 months (61 days) of a transfusion will be excluded from the analysis.

The average changes from baseline in Hb concentrations at Week 16, 20, and 24 in this extension study for Cohort 1 will be summarized. The change from baseline in Hb concentration will also be summarized for all cohorts by visit.

12.3.2.2. Change from Baseline in Markers of Hemolysis and Markers of Erythropoiesis

A summary of actual values and change from corresponding baseline at each visit will be provided. Change in markers of hemolysis will be summarized separately for Cohort 1 by Hb response status.

12.3.2.3. Number of Transfusion Events

The number of transfusion events and number of RBC units transfused will be summarized by cohort based on the SAS and adjusted for the duration on study drug during this extension study. Transfusions received over up to 3 consecutive days will be considered as a single transfusion event.

Additional modeling may be planned and described in detail in the SAP.

12.3.2.4. Patient Reported Outcome-related Endpoints

For PKDD and PKDIA, summary statistics will be provided based on the validated algorithm once available from Study AG348-C-006.

12.4. Safety Analyses

Evaluating the long-term safety and tolerability of AG-348 is the primary objective of this study. The overall safety profile of AG-348 will be assessed in terms of the following:

- Treatment-emergent AEs (TEAEs), including serious TEAEs, AESIs, and TEAEs leading to dose reduction, interruption, and discontinuation
- Clinical laboratory values
- Bone mineral density and its T- and Z-scores
- Vital signs
- ECGs

Only a descriptive analysis of safety will be performed (ie, no formal statistical testing will be performed).

12.4.1. Adverse Events

Treatment-emergent AEs will be summarized based on the SAS.

For analysis purposes, AEs will be classified as TEAEs or posttreatment AEs.

- **TEAE:** any AE that increased in severity or newly developed during the treatment-emergent period.
- **Posttreatment AE:** any AE that increased in severity or newly developed after the treatment-emergent period.

An overview of the TEAE profile will be provided, including the total number of TEAEs, with the number and percentage of subjects for the following categories: (1) All TEAEs, (2) Grades 3/4 TEAEs, (3) TEAEs by relationship to study drug, (4) TEAEs by maximum severity, (5) TEAEs leading to treatment interruption, (6) TEAEs leading to treatment discontinuation, (7) TEAEs leading to dose reduction, (8) Serious TEAEs, (9) Related serious TEAEs, (10) TEAEs leading to death, and (11) AESIs. A summary of the number and percentage of subjects, as well as the number of events per 100 patient-years (number of events adjusted for the total duration of exposure), will be provided.

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

- All TEAEs
- Grades 3/4 TEAEs
- TEAEs by relationship to study drug
- TEAEs by maximum severity
- TEAEs leading to treatment discontinuation
- TEAEs leading to treatment interruption
- TEAEs leading to dose reduction
- Serious TEAEs
- Related serious TEAEs
- TEAEs leading to death
- AESIs

Summaries will be presented by MedDRA SOC and PT using the number and percentages of subjects as well as the number of events per 100 patient-years (number of events adjusted for the total duration of exposure). 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.

Additional summary tables will be presented for TEAEs showing the number and percentage of subjects with any TEAEs by PT.

In addition, 3 listings containing individual subject AE data will be provided: all deaths, SAEs, and permanent discontinuations due to AE.

12.4.2. Laboratory Assessments

A summary of actual values and change from baseline values at each visit will be provided for the continuous laboratory results in SI units based on the SAS.

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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 visits.

12.4.3. Bone Mineral Density Findings

For bone mineral density measured by DXA scans, the actual values, T- and Z-scores, and their change from baseline values will be summarized for each scanned area and at each scheduled assessment.

The number and percentage of subjects with a decrease from baseline in DXA Z-score of >7% will be provided.

A listing will be provided for each subject's bone mineral density and its corresponding T- and Z-scores.

12.4.4. Vital Signs

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

12.4.5. Electrocardiogram Findings

The average of ECG triplicates will be used for each scheduled assessment. A summary of actual values and change from baseline values will be provided at each for the following standard digital ECG measurements: PR, QRS, QT, and QT corrected for heart rate intervals (heart rate-corrected QT interval by Bazett's method [QTcB] and heart rate-corrected QT interval by Fridericia's method [QTcF]), QRS duration, and heart rate.

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

12.4.6. Physical Examination

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

12.5. Pharmacokinetic/Pharmacodynamic Analyses

12.5.1. Pharmacokinetic Analyses

For Cohort 1 only, the plasma pharmacokinetic parameters of AG-348 will be computed using non-compartmental methods based on observed plasma concentrations of the parent and actual sample collection times. Descriptive statistics (ie, n, mean, SD, coefficient of variation, median, minimum, and maximum, geometric mean, and geometric coefficient of variation) will be used to summarize the pharmacokinetic parameters for AG-348.

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CCI [redacted]

12.6. Sample Size and Power Considerations

This is an extension study that plans to enroll subjects from qualifying previous studies (Study AG348-C-006 and Study AG348-C-007) who meet the eligibility requirements for this study. Approximately 96, with up to 116, subjects are potentially eligible to be enrolled in this extension study: approximately 76 subjects from Study AG348-C-006 and approximately 20, with up to 40, subjects from Study AG348-C-007, if eligible. Given the primary safety objective and endpoint, Table 7 provides the 95% exact CIs assuming different observed incidence of AEs.

Table 7: 95% Exact Confidence Intervals Assuming Different Observed Incidences of Adverse Events (N=116)

Observed incidence of Adverse Events n/N (%)	95% Confidence Interval
10/116 (0.09)	(0.04, 0.15)
20/116 (0.17)	(0.11, 0.25)
30/116 (0.26)	(0.18, 0.35)
40/116 (0.34)	(0.26, 0.44)

Abbreviations: n=incidence; N=number of subjects in the study.

13. ADMINISTRATIVE REQUIREMENTS

13.1. Good Clinical Practices

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

13.2. Ethical Considerations

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

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

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

13.3. Subject Information and Informed Consent

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

After the study has been fully explained, written informed consent will be obtained from the subject before 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

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

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

All unused study drug and other study materials 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.

14. LIST OF REFERENCES

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15. APPENDICES

APPENDIX 1. DEFINITION OF WOMEN OF REPRODUCTIVE POTENTIAL

Definition of Women of Reproductive Potential

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

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

1. Premenarchal
2. Premenopausal female with 1 of the following:
   - Documented hysterectomy
   - Documented bilateral salpingectomy
   - Documented bilateral oophorectomy

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

   Documentation can come from the subject's medical records, medical examination, or medical history interview.
3. Postmenopausal female
   - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
     – A high FSH level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormone replacement therapy; however, in the absence of 12 months of amenorrhea, confirmation with >1 FSH measurement is required.


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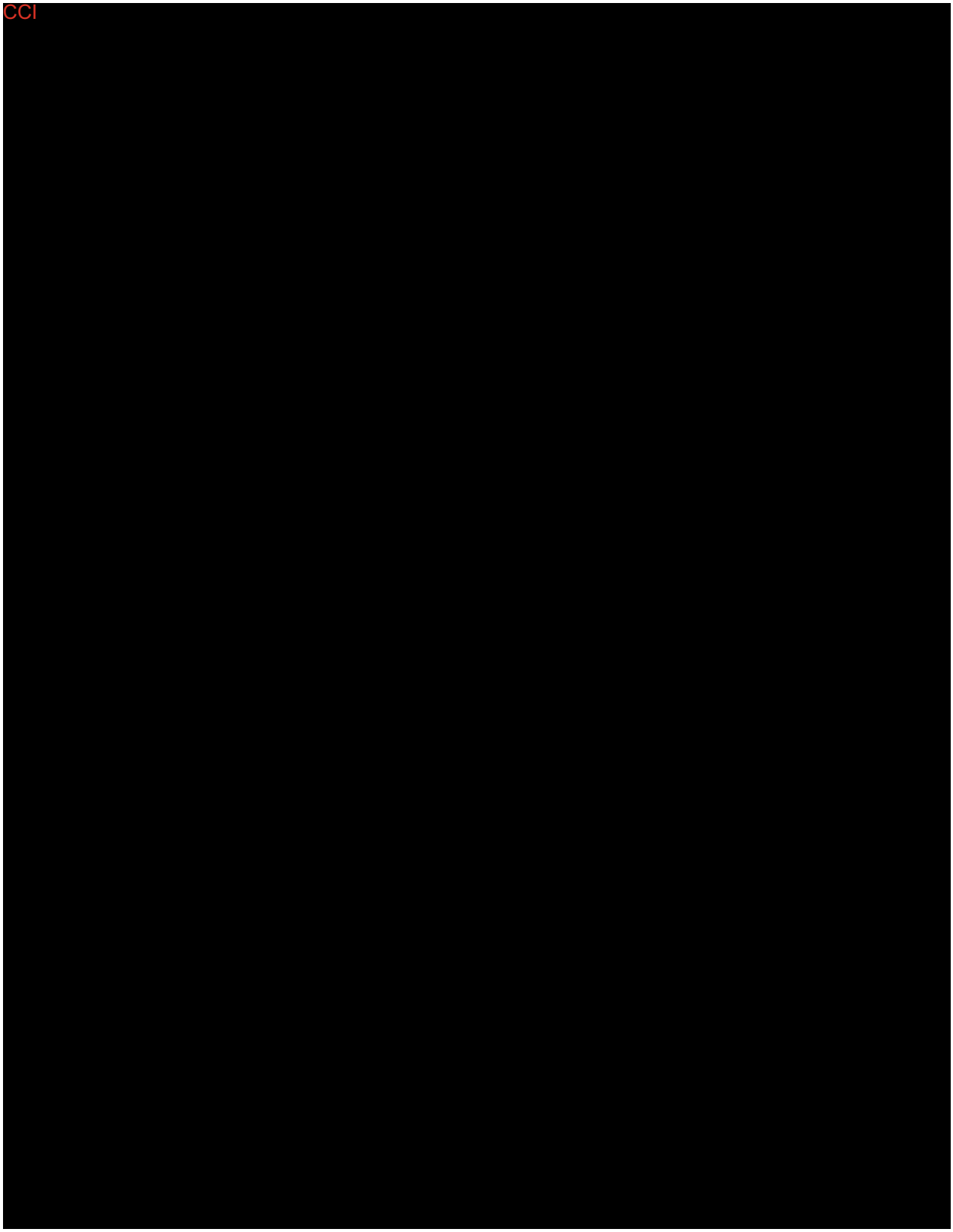
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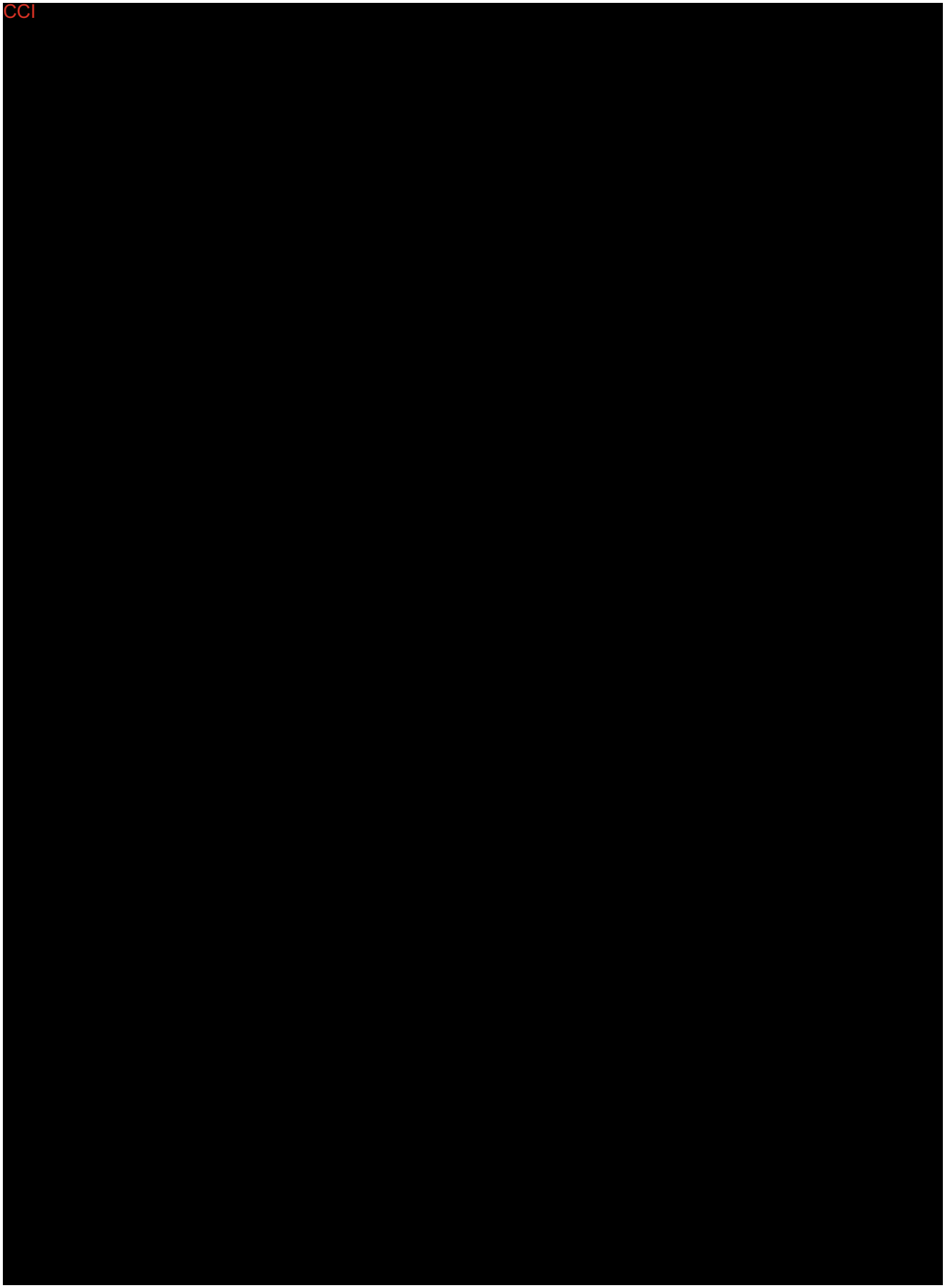
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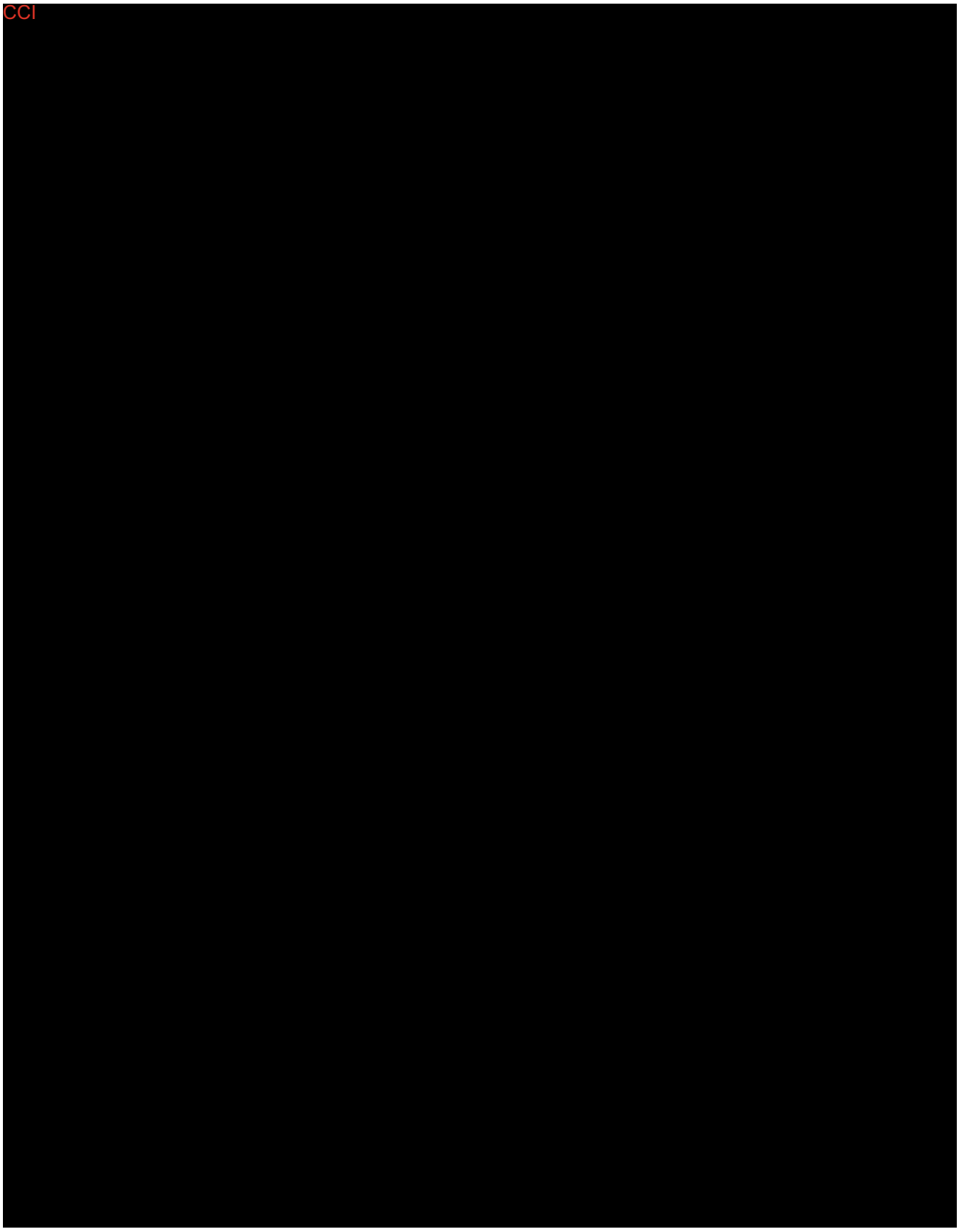
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