CLINICAL STUDY PROTOCOL AG348-C-010

A PHASE 2, OPEN-LABEL, MULTICENTER STUDY TO DETERMINE THE EFFICACY, SAFETY, PHARMACOKINETICS, AND PHARMACODYNAMICS OF AG-348 IN ADULT SUBJECTS WITH NON-TRANSFUSION-DEPENDENT THALASSEMIA

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

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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:

DocuSigned by:
[redacted]
Signer Name: [redacted]
Signing Reason: I approve this document
Signing Time: 27-Aug-2020 | 12:40 PM EDT
45A8610618A3400E93605B3B3FD1E486

[redacted], MD, MS

[redacted]

Signature/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)

PROTOCOL AMENDMENT SUMMARY OF CHANGES

Assessment of Amendment 4 (Version 4.0)

This amendment is considered substantial because it increases the duration of the study and study treatment by an additional 8 years, implements telemedicine visits and direct-to-subject study drug dispensation, adds guidance for allowable modifications to study conduct during declared public health emergencies and natural disasters, and updates guidance regarding concomitant therapies requiring careful monitoring.

The rationale for the protocol amendment is described in the following section; a detailed summary of the amendment changes is provided in a separate summary of changes document.

Purpose and Rationale for the Protocol Amendment

The primary purpose for the protocol amendment was to increase the duration of the Extension Period of the study and update guidance regarding concomitant therapies requiring careful monitoring, in alignment with the AG-348 Investigator's Brochure Version 8.0 (17 June 2020):

- The duration of the Extension Period was increased from 2 to up to 10 years to ensure that subjects who are benefiting from AG-348 may continue treatment while being evaluated for long-term safety and tolerability.
- Moderate inducers of cytochrome P450 (CYP)3A4 were added to the list of therapies that require careful monitoring. Simulations from a physiologically based pharmacokinetic model in Study AG348-C-012 showed there is a potential for drug interactions between AG-348 and moderate inducers of CYP3A4.
- Deferoxamine, deferasirox, and deferiprone, which are iron chelators, were added to the list of therapies that require careful monitoring. In an in vitro study (Report AG348-N-103), AG-348 was found to be a potential inducer of uridine 5′-diphospho-glucuronosyltransferase (UGT)1A1. As a potential UGT1A1 inducer, AG-348 has the potential to reduce the effectiveness of iron chelators metabolized by UGT1A1.

In addition, the following substantial changes were incorporated:

- Telemedicine visits and direct-to-subject shipment of study drug were implemented in the Extension Period to alleviate the burden on subjects associated with on-site study visits.
- Guidance on allowed modifications to study conduct during declared public health emergencies and natural disasters was added for situations during which adherence to protocol-specified procedures is impeded, such as during the COVID-19 pandemic.
- Digoxin and strong inhibitors of P-glycoprotein were removed from the list of therapies that are prohibited, based on simulations from a physiologically based pharmacokinetic model that showed no drug interaction potential between these drugs and AG-348. Note: The eligibility criteria were not amended because study enrollment was completed before the finalization of this amendment.
- Sensitive substrates of CYP2B6 were removed from the list of therapies requiring careful monitoring, based on simulations from a physiologically based pharmacokinetic model that showed no drug interaction potential between AG-348 and sensitive substrates of CYP2B6.

2. SYNOPSIS

Name of Sponsor/Company:
Agios Pharmaceuticals, Inc.

Name of Investigational Product:
AG-348

Study Title:
A Phase 2, Open-Label, Multicenter Study to Determine the Efficacy, Safety, Pharmacokinetics, and Pharmacodynamics of AG-348 in Adult Subjects With Non-Transfusion-Dependent Thalassemia

Study Center(s):
This study will be conducted at multiple study centers in North America and the European Union.

Phase of Development:
2

Objectives:

Primary Objective:

The primary objective of this study is to evaluate the efficacy of treatment with AG-348 in increasing hemoglobin (Hb) concentrations in subjects with non-transfusion-dependent thalassemia (NTDT).

Secondary Objectives:

The following secondary objectives will be assessed in subjects with NTDT:

- To evaluate the safety of AG-348
- To determine the effect of AG-348 on markers of hemolysis and erythropoietic activity
- To evaluate the pharmacokinetics of AG-348

Exploratory Objectives:

- To determine the effect of AG-348 in subjects with NTDT on the following:
  - Pharmacodynamic (PD) markers of thalassemia
  - Other markers of erythropoietic activity
  - Markers of iron metabolism and indicators of iron overload
  - Markers of oxidative stress and other related markers
  - Transfusion burden
  - Spleen size
- To evaluate the relationship between AG-348 pharmacokinetics and indicators of clinical activity in subjects with NTDT
- To evaluate the relationship between the dose of AG-348 and change in Hb concentrations in subjects with NTDT

Study Endpoints:

Primary Endpoint:

The primary endpoint of this study is the Hb response (HR), defined as a ≥1.0 g/dL increase in Hb concentration from baseline at 1 or more assessments between Week 4 and Week 12 (inclusive). An individual subject's baseline Hb concentration is defined as the average of all of the subject's available Hb concentrations during the Screening Period up to the first dose of study drug.

Secondary Endpoints:

- The mean change from baseline in Hb concentrations over a continuous 12-week interval from Week 12 to Week 24
- The sustained Hb response (sHR), defined as a subject who has achieved an HR and has achieved a ≥1.0 g/dL increase in Hb concentration at 2 or more evaluable Hb assessments out of the 4 scheduled assessments between the Week 12 Visit and Week 24 Visit (ie, Weeks 12, 16, 20, and 24)
- The delayed Hb response, defined as a subject who has not achieved an HR, but has achieved a ≥1.0 g/dL increase in Hb concentration at 1 or more Hb assessments after Week 12 (ie, Weeks 16, 20, and 24)
- Change from baseline in Hb concentration over the duration of the Extension Period
- Time to first ≥1.0 g/dL increase in Hb concentration
- Change from baseline in markers of hemolysis: reticulocyte count, bilirubin, lactate dehydrogenase (LDH), and haptoglobin
- Change from baseline in markers of erythropoietic activity: nucleated red blood cell (NRBC), erythropoietin (EPO), and soluble transferrin receptor
- Safety Endpoints:
  - The type, incidence, severity, and relationship to treatment with AG-348 of adverse events (AEs) and serious adverse events (SAEs), AEs of special interest (AESIs), AEs leading to study drug dose reduction, study drug interruption, and study drug discontinuation
  - Changes over time in clinical laboratory tests (serum chemistry, liver function tests (LFTs), LDH, hematology, coagulation, lipids, sex steroids, and urinalysis), physical examination (PE) findings, bone mineral density (BMD) of the hip and lumbar spine, vital signs, and 12-lead electrocardiogram (ECG) findings
- Pharmacokinetic Endpoints:
  - Pharmacokinetic endpoints include drug concentrations over time and pharmacokinetic parameters of AG-348, including area under the plasma concentration × time curve (AUC), maximum (peak) concentration (C_{max}), and others as applicable

Exploratory Endpoints:

- Change from baseline in alpha(α)-, beta(β)-, and gamma(γ)-hemoglobin absolute levels and/or ratios
- Change from baseline in other markers of erythropoietic activity: growth differentiation factor (GDF)-15, GDF-11, and erythroferrone
- Change from baseline in markers of iron metabolism and indicators of iron overload
- Change from baseline in markers of oxidative stress: urinary 8-isoprostane, methylmalonic acid, total homocysteine, and other red blood cell (RBC) metabolite measurements
- Proportion of subjects requiring transfusions and the total number of RBC units transfused
- Change from baseline in spleen size as assessed by magnetic resonance imaging (MRI)
- Change in Hb concentrations in relation to the dose of AG-348
- Pharmacokinetic/PD Endpoints:
  - Change from baseline in adenosine triphosphate (ATP), 2,3 diphosphoglycerate (2,3-DPG) concentrations, RBC-specific form of pyruvate kinase (PKR) activity, PKR protein levels, and PKR flux assay results

– Exposure-response (or pharmacokinetic-PD) relationship between relevant pharmacokinetic parameters and endpoints that are indicators of clinical activity

Methodology:

Overview:

This is a Phase 2, open-label, multicenter study evaluating the efficacy, safety, pharmacokinetics, and PD of treatment with AG-348 in adult subjects with NTDT. This study will consist of a Core Period (up to 24 weeks) followed by an Extension Period (up to 10 years). Approximately 17 subjects with NTDT are planned to be enrolled.

All eligible subjects will receive an initial AG-348 dose of 50 mg twice daily (BID) dosing. At the Week 6 Visit, subjects may undergo an intrasubject dose escalation to 100 mg BID based on an evaluation of the subject's safety and Hb concentrations by the treating Investigator. All dose increases must be reviewed and confirmed by the Medical Monitor (or designee).

Subjects will **not** be permitted to increase to 100 mg BID if they meet either of the following criteria:

- Subject has achieved an Hb concentration increase from baseline to 12 g/dL for female subjects or 13 g/dL for male subjects (inclusive) **or**
- Subject has experienced any Grade 3 or greater treatment-emergent adverse event (TEAE) that is considered related to the study drug.

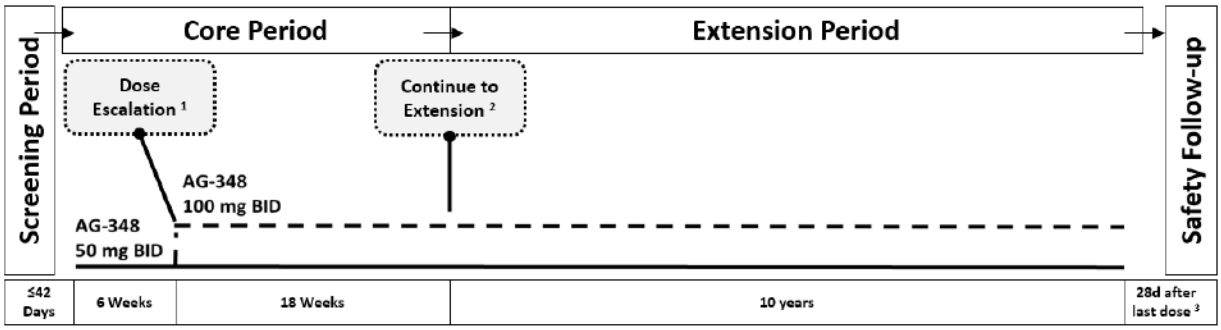
Subjects who meet the following criteria will be permitted to continue study drug in the Extension Period after confirmation by the Medical Monitor (or designee):

- Complete the 24-week Core Period
- Achieve an HR (as defined by the primary endpoint) **or** achieve a delayed HR (defined in the secondary endpoints)
- No ongoing Grade 3 or greater TEAE considered related to study drug

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

An overview of the study design is presented below.

Study Schema



Abbreviations: BID = twice daily; d = days.

[1] Subjects may undergo an intrasubject dose escalation at the Week 6 Visit based on an evaluation of safety and Hb concentration after confirmation by the Medical Monitor (or designee).

[2] Subjects who complete the 24-week Core Period and achieve an HR or a delayed HR with an acceptable safety profile may continue study drug for up to an additional 10 years in the Extension Period, after confirmation by the Medical Monitor (or designee).

[3] The Safety Follow-up Visit will occur 28 days (±4 days) after the subject's last dose of study drug (including taper doses).

Visit Schedule and Analysis Time Points:

During the Core Period, all subjects will attend visits on Day 1, then every 2 weeks for the first 8 weeks, and then every 4 weeks thereafter through Week 24.

During the Extension Period, eligible subjects will continue receiving treatment with AG-348 at the same dose as given at their Week 24 Visit. Study visits will be conducted approximately every 12 weeks for up to 10 years after the subject's Week 24 Visit. At scheduled telemedicine visits, assessments will be collected from subjects remotely by the Investigator or site staff as allowed per local institutional standard of care and regulations; for sites where telemedicine is not permitted by local regulations, subjects are to complete their assessments in person at the site.

Following discontinuation of AG-348, subjects will attend a Safety Follow-up Visit 28 days (±4 days) after their last dose of AG-348 (including taper doses). Subjects with an AE considered to be related to the study drug will continue to be followed.

Number of Subjects Planned:

Approximately 17 subjects with NTDT are planned to be enrolled.

Inclusion/Exclusion Criteria:

Inclusion Criteria:

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

1. Have provided signed written informed consent prior to performing any study procedure, including screening procedures.
2. Be aged 18 years or older.
3. Have a known medical history of thalassemia, including β-thalassemia intermedia, Hb E β-thalassemia, α-thalassemia (Hb H disease), or β-thalassemia with mutations of 1 or more α genes.
4. Have documented clinical laboratory confirmation of thalassemia by Hb electrophoresis/high-performance liquid chromatography (HPLC) or DNA analysis, either from medical records or during the Screening Period.
5. Have an Hb concentration ≤10.0 g/dL, regardless of sex, based on an average of at least 2 Hb measurements (separated by a minimum of 7 days) during the Screening Period.
6. Be considered non-transfusion-dependent, defined as having no more than 5 units of RBCs transfused during the 24-week period up to the first day of study drug **and** no RBC transfusions in the 8 weeks prior to the first day of study drug.
7. Have adequate organ function, as defined by:
   a. Serum aspartate aminotransferase (AST) ≤2.5 × the upper limit of normal (ULN) and alanine aminotransferase (ALT) ≤2.5 × ULN.
   b. Estimated glomerular filtration rate (GFR) ≥60 mL/min/1.73 m^2, measured GFR ≥60 mL/min, or calculated creatinine clearance (CrCL; Cockcroft-Gault) ≥60 mL/min.
   c. Normal levels of serum bilirubin; or if serum bilirubin >ULN, the elevation must not be associated with choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease. Elevated bilirubin attributed to hemolysis with or without Gilbert's syndrome is not exclusionary.
   d. Absolute neutrophil count (ANC) ≥1.0 × 10^9/L.
   e. Platelet count ≥100 × 10^9/L, in the absence of a spleen, or platelet count ≥50 × 10^9/L, in the presence of a spleen and in the absence of any other cause of thrombocytopenia.

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

8. For women of reproductive potential, have a negative serum pregnancy test during the Screening Period and a negative serum or urine pregnancy test on Day 1. Women of reproductive potential are defined as sexually mature women who have not undergone a hysterectomy, bilateral oophorectomy, or tubal occlusion; or who have not been naturally postmenopausal (ie, who have not menstruated at all for at least the preceding 12 months prior to signing informed consent and have an elevated follicle-stimulating hormone (FSH) level indicative of menopause during the Screening Period).
9. For women of reproductive potential as well as men with partners who are women of reproductive potential, be abstinent as part of their usual lifestyle, or agree to use 2 forms of contraception, 1 of which must be considered highly effective, from the time of giving informed consent, during the study, and for 28 days following the last dose of study drug for women and 90 days following the last dose of study drug for men. A highly effective form of contraception is defined as combined (estrogen and progestin containing) hormonal contraceptives (oral, intravaginal, or transdermal) associated with inhibition of ovulation; progestin-only hormonal contraceptives (oral, injectable, or implantable) associated with inhibition of ovulation; intrauterine device; intrauterine hormone releasing system; bilateral tube occlusion; or vasectomized partner. The second form of contraception can include an acceptable barrier method, which includes male or female condoms with or without spermicide, and cervical cap, diaphragm, or sponge with spermicide. Women of reproductive potential using hormonal contraception as a highly effective form of contraception must also utilize an acceptable barrier method while enrolled in the study and for at least 28 days after their last dose of study drug.
10. Be willing to comply with all study procedures for the duration of the study.

Exclusion Criteria:

Subjects who meet any of the following criteria will not be eligible for the study:

1. Have known history of diagnosis of Hb S or Hb C forms of thalassemia.
2. Have a significant medical condition that confers an unacceptable risk to participating in the study, and/or could confound the interpretation of the study data. Such significant medical conditions include, but are not limited to the following:
   a. Poorly controlled hypertension (defined as systolic blood pressure [BP] >150 mmHg or diastolic BP >90 mmHg) refractory to medical management.
   b. History of recent (within 6 months prior to signing informed consent) congestive heart failure; myocardial infarction or unstable angina pectoris; hemorrhagic, embolic or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.
   c. Iron overload sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac (eg, clinically significant impaired left ventricular ejection fraction), hepatic (eg, cirrhosis or severe fibrosis), or pancreatic (eg, diabetes) dysfunction.
   d. Cardiac dysrhythmias judged as clinically significant by the Investigator.
   e. Heart-rate corrected QT interval-Fridericia's method (QTcF) >450 msec (average of triplicate ECGs) with the exception of subjects with right or left bundle branch block.
   f. Liver disease with histopathological evidence of cirrhosis or severe fibrosis.
   g. Clinically symptomatic cholelithiasis or cholecystitis. Prior cholecystectomy is not exclusionary.
   h. History of drug-induced cholestatic hepatitis.

i. Have a diagnosis of any other congenital or acquired blood disorder or any other hemolytic process. Allo-immunization will be allowed unless the subject has an ongoing hemolytic process related to antibodies.

j. Positive test for hepatitis C virus (HCV) antibody (Ab) with evidence of active HCV infection or positive test for hepatitis B surface antigen (HBsAg).

k. Positive test for human immunodeficiency virus (HIV)-1 or -2Ab.

l. Active infection requiring the use of parenteral anti-microbial agents or Grade ≥3 in severity (per National Cancer Institute Common Terminology Criteria for Adverse events [CTCAE] v4.03) within 1 month prior to the first day of study drug.

m. Diabetes mellitus judged to be under poor control by the Investigator or requiring >3 antidiabetic agents, including insulin (all insulins are considered 1 agent); use of insulin per se is not exclusionary.

n. History of any primary malignancy with the exception of: curatively treated nonmelanomatous skin cancer; curatively treated cervical or breast carcinoma in situ; or other primary tumor treated with curative intent, no known active disease present, and no treatment administered during the last 3 years.

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

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

q. Pattern or frequency of post-splenectomy sepsis that, in the assessment of the Investigator, could reasonably be expected to interfere with the ability of the subject to complete the study.

r. Lung disease, including pulmonary fibrosis clinical syndrome or pulmonary hypertension requiring oxygen (O_2) therapy.

s. Pulmonary hypertension with tricuspid regurgitation velocity (TRV) ≥3.2 m/s by echo Doppler (obtained within 6 months of Screening).

t. Proteinuria >2 + on dipstick.

u. Clinical diagnosis of osteoporosis (ie, a T-score of −2.5 or lower at the lumbar spine, femur neck, or total hip by bone mineral density testing).

v. Grade ≥3 triglyceride elevations.

3. Have a splenectomy scheduled during the study treatment period or have undergone splenectomy within 12 months prior to signing informed consent.
4. Are currently enrolled in another therapeutic clinical trial involving ongoing therapy with any investigational or marketed product or placebo.
5. Have exposure to any investigational drug, device, or procedure within 3 months prior to the first day of study drug.
6. Have prior exposure to sotatercept (ACE-011), luspatercept (ACE-536), ruxolitinib, or gene therapy.
7. Have a prior bone marrow or stem cell transplant.
8. Are currently pregnant or breastfeeding.
9. Have a history of major surgery within 6 months of signing informed consent. Note that procedures such as laparoscopic gallbladder surgery are not considered major in this context.
10. Are currently receiving medications that are strong inhibitors of cytochrome P450 (CYP)3A4, strong inducers of CYP3A4, strong inhibitors of P-glycoprotein (P-gp), or digoxin (a P-gp

sensitive substrate medication) that have not been stopped for a duration of at least 5 days or a timeframe equivalent to 5 half-lives (whichever is longer) prior to the first day of study drug.

11. Are currently receiving chronic anticoagulant therapy, unless started and on a stable dose for at least 28 days prior to first day of study drug.
12. Are currently receiving anabolic steroids, including testosterone preparations, if initiated ≤28 days prior to the first day of study drug.
13. Are currently receiving hematopoietic stimulating agents (eg, erythropoietins, granulocyte colony stimulating factors, thrombopoietins), if initiated ≤8 weeks prior to the first day of study drug.
14. Have a history of allergy to sulfonamides if characterized by acute hemolytic anemia, drug-induced liver injury, anaphylaxis, rash of erythema multiforme type or Stevens-Johnson syndrome, cholestatic hepatitis, or other serious clinical manifestations.
15. Have a history of allergy to AG-348 or its excipients (microcrystalline cellulose, croscarmellose sodium, sodium stearyl fumarate, and mannitol).

Investigational Product, Dosage, and Mode of Administration:

AG-348 will be administered orally BID as a 50 mg tablet.

The initial dose of AG-348 will be 50 mg BID with 1 potential dose-level increase (ie, from 50 to 100 mg BID) at the Week 6 Visit.

Duration of Study:

Duration of Study Treatment:

The maximum duration of study treatment for all subjects will be approximately 10.4 years (542 weeks) including 24 weeks in the Core Period, 516 weeks in the Extension Period (for eligible subjects), and up to 2 weeks for the final dose taper.

Duration of Study Participation

A subject's maximum duration of study participation is approximately 10.5 years (552 weeks) from Screening to the Safety Follow-up Visit.

End of Study:

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

Criteria for Evaluation:

Efficacy: Hb, RBC parameters, haptoglobin, bilirubin, and LDH

Safety: AEs, concomitant medications and non-drug therapies, PE findings, vital signs, ECG findings, BMD, and clinical laboratory assessments

Pharmacokinetics: Trough and full pharmacokinetic profile

Pharmacodynamics: ATP, 2,3-DPG, PKR protein, PKR activity, and PKR flux

Other: Iron-related markers, liver iron concentration and spleen size as assessed by MRI, markers of oxidative stress, markers of erythropoietic activity, globin chain quantitation, transfusion records, and markers of hemolysis

Sample Size:

With a total of 17 subjects enrolled, the study will have 80% power to reject a 30% null response rate at a one-sided 0.05 type I error rate when the true response rate is 60%.

Statistical Methods:

The study data will be analyzed and reported in the primary clinical study report when all subjects have completed the Week 24 Visit or Safety Follow-up Visit (if applicable) or have discontinued the study. A detailed analysis plan for the Core Period efficacy and safety data will be presented in a statistical analysis plan (SAP), which will be finalized before the Core Period database lock.

Once all subjects have completed the Extension Period and Safety Follow-up Visit (as applicable) or have discontinued the study, additional data will be analyzed for long-term safety and efficacy. The analysis will be specified in a SAP addendum or a separate SAP as considered appropriate, which will be finalized before the final database lock.

Analysis Sets:

- Full Analysis Set (FAS): All subjects who receive at least 1 dose of study drug.
- Safety Analysis Set: All subjects who receive at least 1 dose of study drug. In this nonrandomized study, the FAS and the Safety Analysis Set are identical.

General Methods:

All data will be presented in by-subject listings. Categorical variables will be summarized by frequency distributions (ie, number and percentages) and continuous variables will be summarized by descriptive statistics (ie, mean, standard deviation [SD], median, minimum, maximum). Study data will be summarized for disposition, demographics, and baseline disease characteristics.

Primary Efficacy Analysis:

In the primary analysis, number and percentage of subjects with an HR will be summarized based on FAS, along with the 90% confidence interval (CI) based on exact distribution. If the lower bound of the 90% CI is greater than 30%, the null response rate will be rejected. For subjects who discontinue study drug before Week 4, Hb increase at the last available Hb assessment will be imputed (ie, last observation carried forward [LOCF]) and will be used to evaluate the subject's Hb responder status. To evaluate the impact of missing data and LOCF, sensitivity analyses will be conducted to only include subjects who have at least 1 assessment between the Week 4 and the Week 12 Visits (inclusive).

In the analysis of Hb, Hb results within 8 weeks of transfusion will be excluded from the analysis.

Secondary Efficacy Analyses:

Mean Change from Baseline from Week 12 to Week 24: Average change from baseline in Hb concentrations from Week 12 to Week 24 will be calculated for each subject using all Hb concentrations collected within the Week 12 to Week 24 windows and will be summarized as a continuous variable. In addition, average change from baseline in Hb concentrations from Week 4 to Week 6, Week 8 to Week 12, Week 16 to Week 24, Week 4 to Week 24, and Week 4 to Week 12 for all subjects in the FAS as well as Hb responders will be summarized descriptively.

Sustained Hb Response: The number and percentage of subjects with sHR will be summarized, along with its 95% CI based on exact distribution. The analysis will be based on FAS. Subjects with ≤1 Hb assessment at Week 12, Week 16, Week 20, and Week 24 will be considered not reaching the sHR. Similar analysis will be repeated including subjects with at least 2 Hb assessments at Weeks 12, 16, 20, and 24.

Delayed Hemoglobin Response: The number and percentage of subjects with a delayed Hb response will be summarized, along with its 95% CI based on exact distribution.

Change from Baseline in Hemoglobin Concentration Over the Duration of the Extension Period: In the summary of Hb concentrations and Hb change from baseline at each visit, all available data including those on or after Week 24 visit will be included. However, as only Hb responders and delayed responders with an acceptable safety profile may continue AG-348 treatment in the Extension Period,

data from the visits in the Extension Period are only reflective of these responders and need to be interpreted with caution.

Time to First ≥1.0 g/dL Increase in Hemoglobin Concentration from Baseline: Days to first ≥1.0 g/dL increase in Hb concentration from baseline will be summarized as a continuous variable as well as in categories (<6, 6 to <12, ≥12 weeks) for Hb responders.

Changes from Baseline in Markers of Hemolysis and Markers of Erythropoietic Activity: At each visit this will be summarized and plotted by Hb responder status.

Safety Analysis:

Safety will be summarized using descriptive statistics. Summaries will be produced for all TEAEs, related TEAEs, serious TEAEs, TEAEs leading to study drug discontinuation, TEAEs Grade ≥3 in severity, and AESIs. Individual subject listings will be provided for any deaths, SAEs, and TEAEs leading to study drug interruption or discontinuation.

For clinical laboratory values, vital signs, BMD of the hip and lumbar spine, and ECG findings, both actual values and changes from baseline will be summarized by visit using summary statistics. The number and percentage of subjects with transaminase increases of >2.5× baseline or an increase in AST or ALT to Grade ≥2 (AESI of transaminase increase as defined in the body of the protocol), will be summarized.

Pharmacokinetic and PD Analysis:

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 (such as 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.

Descriptive statistics will be used to summarize PD parameters for the entire population. Pharmacodynamic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation, median, minimum and maximum, geometric mean, and geometric coefficient of variation.

If sufficient data are obtained, an exposure-response analysis may be performed to evaluate the relationship of pharmacokinetics of AG-348 with PD parameters and indicators of efficacy or clinical activity. If such an analysis is conducted, it could be described and conducted under a separate analysis plan.

Interim Analysis and Independent Data Monitoring Committee (IDMC):

No interim analysis or IDMC is planned.

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4. ABBREVIATIONS

Abbreviation	Definition
λ_z	Apparent terminal elimination rate constant, calculated from a semi-log plot of the plasma concentration versus time curve
α	Alpha
Ab	Antibody
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
$AUC_{0-\tau}$	Area under the plasma concentration × time curve from 0 to the end of the dosing period
β	Beta
BID	Twice daily
BP	Blood pressure
CI	Confidence interval
C_{max}	Maximum (peak) concentration
CrCL	Creatinine clearance
CTCAE	Common Terminology Criteria for Adverse Events
CYP	cytochrome P450 enzymes
2,3-DPG	2,3-Diphosphoglycerate
DXA	Dual-energy X-ray absorptiometry
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
EPO	Erythropoietin
FAS	Full Analysis Set
FSH	Follicle-stimulating hormone
γ	Gamma
GCP(s)	Good Clinical Practice(s)
GDF	Growth differentiation factor
GFR	Glomerular filtration rate

Abbreviation	Definition
Hb	Hemoglobin
HBsAg	Hepatitis B surface antigen
hCG	Human chorionic gonadotropin
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HR	Hemoglobin response
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
LDH	Lactate dehydrogenase
LFT	Liver function test
LOCF	Last observation carried forward
MAD	Multiple ascending dose
MCV	Mean corpuscular volume
MRI	Magnetic resonance imaging
NCI	National Cancer Institute
NTBI	Non-transferrin bound iron
NTDT	Non-transfusion-dependent thalassemia
PD	Pharmacodynamic(s)
PE	Physical examination
PEP	Phosphoenolpyruvate
P-gp	P-glycoprotein
PK deficient/cy	Pyruvate kinase deficient/cy
PKL	Liver-specific form of pyruvate kinase
PKM	Pyruvate kinase muscle isozyme
PKR	Red blood cell-specific form of pyruvate kinase
QTcF	Heart rate-corrected QT interval by Fridericia's method
RBC	Red blood cell
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan

Abbreviation	Definition
SD	Standard deviation
TDT	Transfusion-dependent thalassemia
TEAE	Treatment-emergent adverse event
UGT	Uridine 5′-diphospho-glucuronosyltransferase
ULN	Upper limit of normal
WT	Wild-type

5. INTRODUCTION

5.1. Thalassemia

Inherited hemoglobin (Hb) disorders can be divided into 2 main groups. The first group includes structural Hb variants, such as Hb S, C, and E. The second group includes the alpha(α)- and beta(β)-thalassemias, which result from the defective synthesis of the α- or β-globin chains of adult Hb A (Musallam et al, 2013).

The thalassemias are a group of disorders in which the normal ratio of α- to β-globin production is disrupted due to a disease-causing variant in 1 or more of the globin genes. Alpha-globin aggregates (as found in β-thalassemia) readily precipitate, which disrupts the red blood cell (RBC) membrane and results in oxidative stress. Beta-globin tetramers (Hb H, found in α-thalassemia) are generally more soluble, but are still unstable and can form precipitates. The imbalance of the globin chain synthesis leads to a net reduction in Hb concentrations and has dramatic effects on the survival of RBC precursors, ultimately resulting in their premature destruction in the bone marrow and in extramedullary sites (Cappellini et al, 2014).

5.1.1. Non-Transfusion-Dependent Thalassemia

Transfusion dependence has been an essential factor in characterizing the various thalassemia phenotypes and their severity because the hallmarks of the disease are ineffective erythropoiesis, peripheral hemolysis, and subsequent anemia. The thalassemia syndromes have been reclassified based on transfusion requirements as transfusion-dependent thalassemia (TDT) and non-transfusion-dependent thalassemia (NTDT).

Patients with NTDT are usually diagnosed because of chronic anemia with Hb concentrations between 7 and 10 g/dL. This may be sustainable without the need for regular transfusion therapy, but can lead to significant complications due to the chronic anemia (Taher et al, 2015).

In NTDT, erythropoiesis is ineffective due to the imbalance in the production of α- and β-globin chains. The clinical implications of the α- and β-globin imbalance are 2-fold: 1) patients lack sufficient RBCs and Hb to effectively transport oxygen throughout the body; and 2) increased hemolysis can lead to hypersplenomegaly, bone marrow expansion (extramedullary hematopoiesis), concomitant bone deformities, and iron overload (Galanello and Origa, 2010).

While the symptoms of NTDT may not present as dramatic or life-threatening compared with those in patients with TDT, years of chronic anemia can lead to widespread organ damage and dysregulated compensatory mechanisms. Any of the symptomatic complications of TDT can be observed in patients with NTDT, and are not often treated with transfusions. Specific additional clinical complications of NTDT include cerebral ischemia, pulmonary hypertension, right-sided heart failure, hepatic fibrosis, cirrhosis, hepatocellular carcinoma, extramedullary hematopoietic pseudotumors, gall stones, osteoporosis, folic acid deficiency, infections, and venous thrombosis (Musallam et al, 2013; Taher et al, 2015). Morbidity in patients with NTDT is directly proportional to the severity of ineffective erythropoiesis and peripheral hemolysis (Musallam, Taher, et al, 2011).

5.1.2. Treatment Options for Non-Transfusion-Dependent Thalassemia

The currently available treatment options for NTDT are primarily supportive, therefore, medical management of NTDT takes a more "symptom-focused" approach by treating symptoms as they arise. Symptomatic marrow expansion is treated with hydroxyurea, transfusion, or radiation therapy; endocrine dysfunction is treated with hormone replacement therapies, vitamin D, bisphosphonates, and calcium supplementation; leg ulcers are treated with hydroxyurea plus erythropoietin (EPO) or platelet-derived growth factor (PDGF), hygiene, and transfusions; and thrombotic events are treated with anticoagulation therapies (Karimi et al, 2014).

While the current standard of care for patients with TDT includes regular blood transfusion and iron chelation therapy, blood transfusions are not part of the routine treatment strategy for patients with NTDT, but may be required in the event of infection, pregnancy, or surgery (Musallam et al, 2013). Splenectomy may be useful for patients with more severe forms of NTDT, as it is associated with an increase in Hb concentrations and improvement in growth and development (Karimi et al, 2014).

Removal of excess iron is essential in patients with NTDT with iron overload. Iron overload is frequently derived from transfusion therapy, but can also be caused by chronic hemolysis, ineffective erythropoiesis, and hypoxia, leading to increased intestinal iron absorption through suppression of the regulatory protein hepcidin (Taher et al, 2013). While iron accumulation in patients with NTDT is slower than that observed in patients with TDT, it is a cumulative process that eventually leads to excess iron levels that are clinically significant and may impact overall survival (Musallam, Cappellini, et al, 2011).

5.1.3. Biochemistry of Thalassemia

Studies of RBCs from patients with β-thalassemia intermedia have shown that the adenosine triphosphate (ATP) levels in β-thalassemic RBCs are significantly lower than that found in healthy controls, with a possible functional shift towards increased pentose phosphate pathway (Chakraborty et al, 2012; Ting et al, 1994). Thalassemic cells are more susceptible to loss of ATP and intracellular potassium (K^+) (Gunn et al, 1972) and show increased passive calcium (Ca^{2+}) uptake (Wiley, 1981) after glucose depletion. These observations have been proposed to be related to effects of protein aggregation and oxidative stress on the RBC membrane. It has also been observed that thalassemic RBCs clear excess α-globin chains through ATP-dependent proteolytic processes (Khandros et al, 2012; Shaeffer, 1988).

Collectively, these observations suggest that the imbalance in α/β globin chains in thalassemic RBCs may ultimately lead to a compensatory increased energetic demand. This leads to the hypothesis that enhancement of glycolysis, which is the pathway by which nearly all RBC ATP is derived, may help improve thalassemic RBC survival in the bone marrow and/or peripheral circulation.

5.2. AG-348

5.2.1. Proposed Mechanism of Action of AG-348

AG-348 is an orally available, potent, broad-spectrum activator of the RBC-specific form of pyruvate kinase (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. Pyruvate kinase enzymatically catalyzes the metabolic conversion of phosphoenolpyruvate (PEP) and adenosine diphosphate (ADP) into pyruvate and ATP as the final step in glycolysis. Mature RBCs rely almost exclusively on the process of glycolysis to generate the energy carrier molecule ATP. Thus, PKR is a key enzyme for maintaining energy homeostasis in erythrocytes.

AG-348 acts by directly binding to the PKR tetramer and allosterically enhancing the affinity for its substrate, PEP. Pharmacology studies have confirmed the potency of AG-348 in activating wild-type (WT) and mutant PKR enzyme activity and modulating ATP and 2,3-diphosphoglycerate (2,3-DPG) levels in healthy adult subjects. In preclinical studies, AG-348 has also been shown to have acceptable absorption, distribution, metabolism, and excretion (ADME) and toxicology profiles.

As described in Section 5.1, RBCs in patients with thalassemia suffer from an imbalance in the ratio between the α- and β-chains of Hb. This has the potential to impose several forms of metabolic stress on the cells, specifically in the form of excess generation of reactive oxygen species (ROS) and an increased demand of ATP-dependent proteolytic mechanisms to clear excess globin chains. This leads to the hypothesis that increased ATP synthesis through activation of PKR by AG-348 in thalassemic red cells may result in increased cell survival in the bone marrow or peripheral circulation.

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

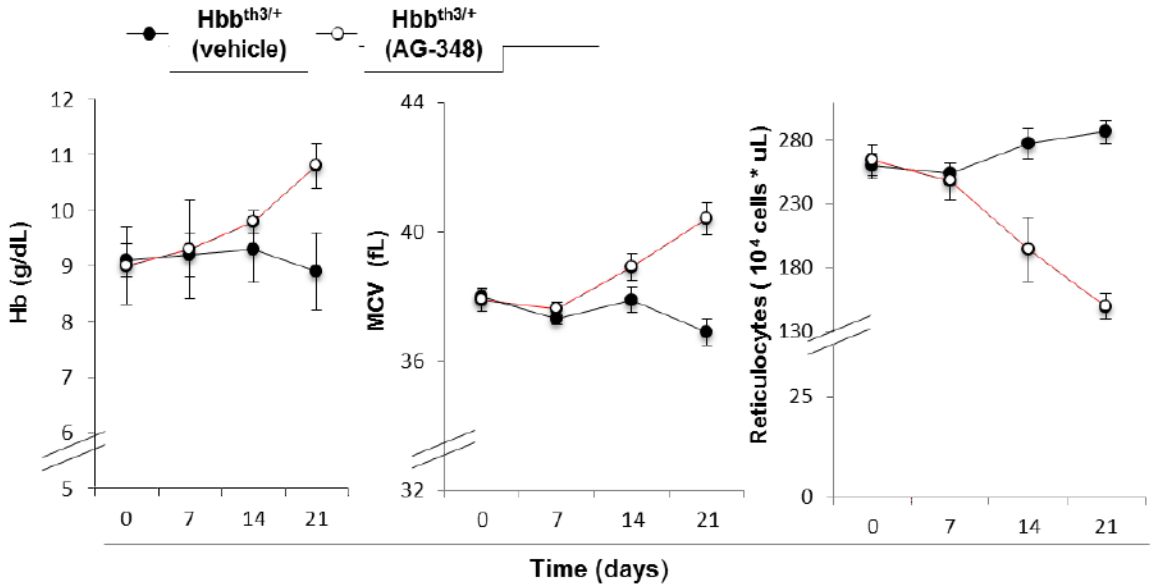
The effect of AG-348 was studied in a mouse model of β-thalassemia, the Hbb^{th3} heterozygote model (abbreviated $Hbb^{th3/+}$ or th3/+), which is widely considered to be comparable to patients with β-thalassemia intermedia in terms of symptoms and severity (McColl and Vadolas, 2016). Mice were treated with AG-348 (50 mg/kg twice daily [BID] via oral gavage or similar total dose through direct addition of AG-348 into mouse chow), and their hematological parameters were evaluated. Preliminary results from this study are presented within this section. Mice treated with AG-348 had increased Hb of approximately 2 g/dL after 21 days of treatment, an increased mean corpuscular volume (MCV), and reduction in reticulocyte counts (Figure 1). As reticulocytes are larger than mature erythrocytes, the increase in MCV with decreased reticulocyte counts is notable and suggests amelioration of the microcytic character of the thalassemic cells. These effects were sustained upon dosing of AG-348 up to 2 months (data not shown).

Adenosine triphosphate levels appeared to be increased in the peripheral blood of mice treated with AG-348 (Figure 2, Panel A). The imbalance in α-β Hb chains with concomitant aggregation of excess α-globin chains is responsible for the underlying pathology of β-thalassemia. The α-β ratio of Hb was significantly improved after treatment with AG-348 (as shown in the Coomassie stain in Figure 2, Panel B), and the soluble Hb fraction was also increased (Figure 2, Panel C), suggesting reduction in α-globin aggregation. Cells from the bone marrow and spleen were collected and analyzed via flow cytometry for staining for annexin V, a marker of apoptosis. AG-348 treatment resulted in a reduction in annexin V positive cells in both tissues (Figure 3, Panel A).

Red cell precursors were sorted from the bone marrow (Chen et al, 2009) of mice treated with vehicle or AG-348 and treated with a fluorescent dye to examine the levels of ROS in these cells. Elevated levels of ROS (compared with WT mice) observed in the β-thalassemic cells were significantly reduced after AG-348 treatment (Figure 3, Panel B). Red cell lifespan in the periphery was also increased following AG-348 treatment (data not shown).

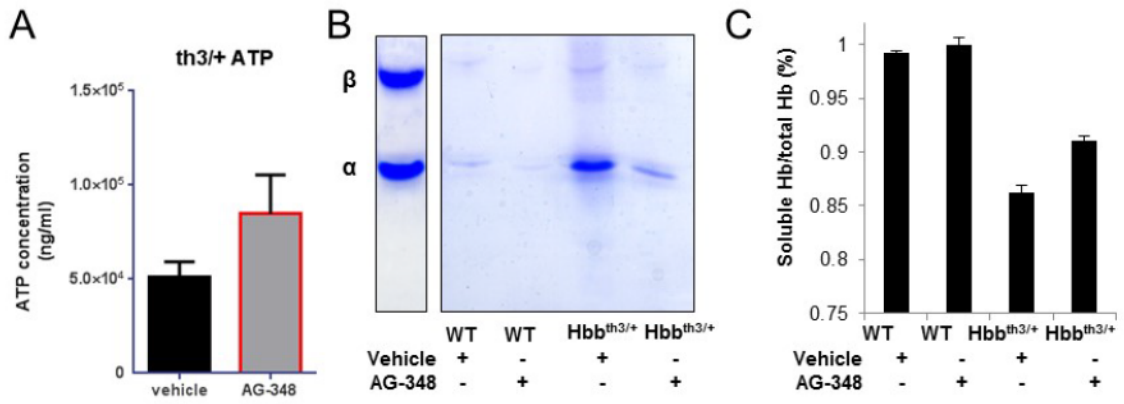
Collectively, these preliminary data indicate that treatment with AG-348 is associated with improved ineffective erythropoiesis and reduced extramedullary erythropoiesis in a mouse model of β-thalassemia, although studies to further understand the mechanisms are underway.

Figure 1: Effect of AG-348 on RBC Hematologic Parameters in Hbb$^{th3/+}$ Mouse Model of Beta-Thalassemia



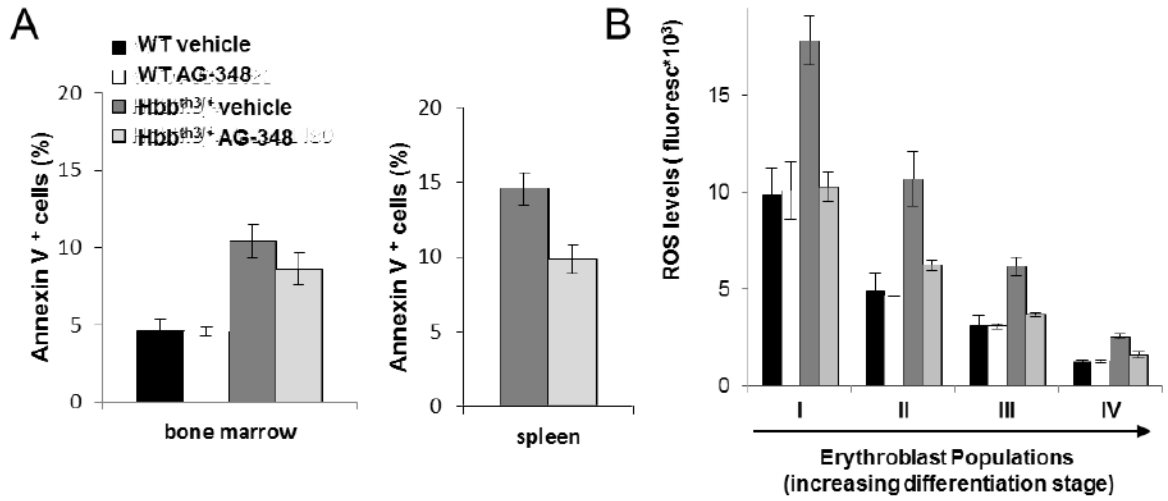
Source: Preliminary results.
Abbreviations: Hb = hemoglobin; Hbb$^{th3/+}$ = heterozygote mouse model of β-thalassemia; MCV = mean corpuscular volume; RBC = red blood cell.

Figure 2: Effect of AG-348 on RBC ATP Levels, Alpha-Beta Hemoglobin Ratio, and Soluble Hemoglobin in Hbb$^{th3/+}$ Mouse Model of Beta-Thalassemia



Source: Preliminary results.
Abbreviation: ATP = adenosine triphosphate; Hb = hemoglobin; Hbb$^{th3/+}$ (or th3/+) = heterozygote mouse model of βthalassemia; RBC = red blood cell; WT = wild type.

Figure 3: Effect of AG-348 on Marker of Apoptosis and Reactive Oxygen Species in Bone Marrow and Spleen of Hbb$^{th3/+}$ Mouse Model of Beta-Thalassemia



Source: Preliminary results.
Abbreviation: Hbb$^{th3/+}$ = heterozygote mouse model of βthalassemia; ROS = reactive oxygen species; WT = wild type.

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 pyruvate kinase deficiency (PK deficiency). Further development in PK deficiency is currently ongoing with 2 pivotal studies in adult

subjects with PK deficiency, 1 in subjects who are not regularly transfused (AG348-C-006), and 1 in subjects who are regularly transfused (AG348-C-007).

An overview of AG-348 pharmacokinetic and pharmacodynamic (PD) data is provided in Section 5.2.3.1 and Section 5.2.3.2, respectively. A brief 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 Investigator's Brochure (IB) for additional details on all clinical studies and results.

5.2.3.1. Summary of AG-348 Pharmacokinetics

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

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

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

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

5.2.3.2. Summary of AG-348 Pharmacodynamics

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

A simple E_{max} (maximum effect) model was used to describe the pharmacokinetic/PD correlation between plasma AG-348 $AUC_{0\text{-}12}$ and blood $AUC\_Net\_B_{0\text{-}12}$ for ATP and 2,3-DPG using results

from the MAD study. The estimated plasma AG-438 $AUC_{0-\tau}$ that resulted in a 50% maximal effect over the dosing interval at steady-state ($EAUC_{50}$) was estimated to be 1,079 ng•h/mL. This corresponded to the total exposure ($AUC_{0-\tau}$) achieved between doses of 15 and 60 mg AG-348 q12h in the MAD study. Based on the observed values of ATP blood AUC_Net_B_{0-12} and the predicted value of the maximum stimulatory effect of AG-348 on ATP blood AUC_Net_B_{0-12} of 1,728 µg•h/mL, approximately 85% of the maximum stimulatory effect was achieved at a dose of 60 mg q12h in the MAD study and over 96% of the maximum stimulatory effect was achieved at a dose of 360 mg q12h in the MAD study. The estimated plasma AG-348 $AUC_{0-\tau}$ that resulted in 50% inhibitory effect ($IAUC_{50}$) on 2,3-DPG over the dosing interval at steady-state was estimated to be 750 ng•h/mL. This corresponded to the AG-348 plasma total exposure ($AUC_{0-\tau}$) achieved following multiple doses of AG-348 below or near 15 mg q12h in the MAD study. Based on the mean observed values of 2,3-DPG blood AUC_Net_B_{0-12} and the predicted value of the maximum inhibitory effect of AG-348 on 2,3-DPG blood AUC_Net_B_{0-12} of 3,381 µg•h/mL, approximately 74% of the maximum inhibitory effect was achieved at a dose of 60 mg q12h in the MAD study and over 99% of the maximum inhibitory effect was achieved at a dose of 360 mg q12h in the MAD study.

Additionally, 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 been evaluated. In this study, no consistent pattern of decrease in concentration of 2,3-DPG or increase in ATP in subjects with or without an Hb response has been observed. The reason for this is not completely clear. However, it is possible these measurements are confounded by changes in the RBC composition (eg, increased hematocrit, reduced reticulocyte count) in responding subjects.

5.2.3.3. Summary of AG-348 Clinical Safety Data

Overall, AG-348 has been generally well tolerated among healthy adult subjects and adult subjects with PK deficiency. Important identified risks associated with administration of AG-348 in clinical studies include bone mineral density (BMD) decrease (including osteoporosis and osteopenia due to aromatase inhibition), withdrawal hemolysis, and insomnia (not clinically serious, ie, not Grade 3 or Grade 4).

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

5.2.3.4. Summary of AG-348 Efficacy Data in PK Deficiency

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 (50.0%) subjects achieved maximum increases in Hb >1 g/dL (Grace et al, 2017). 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. Decreased haptoglobin, reticulocyte/erythrocyte ratio, bilirubin, and lactate

dehydrogenase (LDH) in responders provide additional evidence of the ability of AG-348 to decrease hemolysis, while available flux data support the hypothesis that these beneficial pharmacological and clinical effects are based on activation of pyruvate kinase enzyme. Additionally, Investigators have provided anecdotal reports of responding subjects feeling more energetic, engaging in more exercise, and being better able to perform their daily tasks.

5.3. Study Rationale

A serious unmet medical need exists for patients with NTDT. Treatments for NTDT are limited and primarily supportive, addressing either symptoms (eg, blood transfusions to treat anemia) or complications of the underlying disease or its treatment (eg, chelation therapy for iron overload) and do not address the underlying cause of the disease. Treatment with AG-348 has the potential to modify the underlying pathology of thalassemia by enhancing the energy metabolism of RBCs, leading to improved overall cell fitness and lifespan, reduction of hemolysis, and a sustained increase in functional Hb.

The totality of the preliminary data from the clinical development of AG-348 in PK deficiency and the 2 completed Phase 1 SAD and MAD studies in healthy adult subjects, combined with the nonclinical β-thalassemia mouse model data, supports this study of AG-348 for the treatment of patients with NTDT.

5.3.1. Rationale for Proposed Patient Population

While PKR activation has the potential to treat many forms of thalassemia, this initial study is focused on NTDT patients due to the observed activity of AG-348 in a mouse model of thalassemia that is characterized by symptoms that strongly parallel human NTDT patients.

There is strong agreement within the β-thalassemia medical community that the classification of NTDT includes the forms β-thalassemia intermedia, Hb E β-thalassemia, and β-thalassemia with one or more α genes. The clinical treatment of patients with these forms is essentially identical and includes monitoring of symptoms, elective transfusions as appropriate, and chelation therapy for iron overload. Deletional and non-deletional mutations in the α-chain of Hb can result in a form of α-thalassemia called Hemoglobin H (Hb H) disease, which can result in symptomatic anemia that is managed similarly.

The differential solubility of excess α or β Hb chains results in differences in the clinical profiles between patients with α and β-thalassemia. However, the Hb cutoff in a study should ensure a relatively uniform clinical presentation and is likely to select for patients that have a similar underlying biological dysfunction with respect to protein aggregation (Harewood and Bhimji, 2018). Thus, it is reasonable to expect that the response to a PKR activator will be similar in all patients with these forms of thalassemia.

Patients with a diagnosis of Hb S or Hb C forms of thalassemia are not eligible for this study, to prevent any confounding effects resulting from these mutations. Patients with the Hb C form are excluded from this study as the disease typically has a milder presentation overall and this point mutation in Hb may result in less imbalance between α- and β-globin chains, and thus less protein aggregation. The Hb S form presents with symptoms of moderate anemia and signs of sickle cell anemia, which are usually less frequent and less severe than those of sickle cell

disease. However, because standard-of-care treatment is the same as for sickle cell disease, these patients are excluded from this trial.

5.3.2. Rationale for the Dose Selected

Red blood cells from patients with β-thalassemia intermedia have significantly lower ATP levels than that found in healthy controls, with thalassemic RBC being more susceptible to loss of ATP, likely due to disruptive effects on the RBC membrane from protein aggregation and oxidative stress (Chakraborty et al, 2012; Gunn et al, 1972; Ting et al, 1994). It has also been observed that thalassemic RBCs clear excess α-globin chains through ATP dependent proteolytic processes (Khandros et al, 2012; Shaeffer, 1988). These observations led to the hypothesis that activation of PKR through AG-348 may lead to increased ATP generation and may help improve β-thalassemic RBC survival in the bone marrow or peripheral circulation (Section 5.1). In a nonclinical study in a mouse model of β-thalassemia, ATP levels increased in the peripheral blood of mice treated with AG-348, accompanied with increases in Hb of approximately 2 g/dL after 21 days of treatment (Section 5.2.2).

Patients with thalassemia have WT PKR enzyme as found in healthy individuals. Therefore, pharmacokinetic/PD results from the MAD study (Section 5.2.3.1) conducted in healthy adult subjects were used to select the dose for this study. The simple E_{max} model used to describe the pharmacokinetic/PD correlation between plasma AG-348 $AUC_{0\text{-}12}$ and blood AUC_Net_$B_{0\text{-}12}$ for ATP observations from the MAD study showed that approximately 85% of the maximum stimulatory effect was achieved at a dose of 60 mg BID and over 96% of the maximum stimulatory effect was achieved at a dose of 360 mg BID (Section 5.2.3.1). The initial dose that will be administered to adult subjects with NTDT in this study is 50 mg BID and is expected to result in approximately 85% of the maximum stimulatory effect on ATP. The dose of 50 mg BID, previously studied in adult subjects with PK deficiency, resulted in meaningful changes in Hb concentrations in the PK deficiency patient population (Section 5.2.3.4) and was well-tolerated. Based on the clinical effects of AG-348 on ATP in healthy adults and AG-348 on Hb in adult subjects with PK deficiency, the selected dose of 50 mg BID of AG-348 is expected to increase ATP sufficiently such that it results in meaningful increases in Hb in adult subjects with NTDT.

Adult subjects with NTDT who tolerate AG-348 at 50 mg BID, but have not demonstrated an increase in Hb (as defined in Section 7.3), may be eligible to have their dose escalated to 100 mg BID. Based on clinical studies conducted in healthy adults and in adult subjects with PK deficiency, 100 mg BID of AG-348 is expected to be safe and tolerable. The eligibility for intrasubject dose escalation will be determined by the treating Investigator and Medical Monitor (or designee) and will be based on an evaluation of safety and Hb concentrations. Please refer to Section 7.3 for further details and criteria regarding intrasubject dose escalation.

6. STUDY OBJECTIVES AND ENDPOINTS

6.1. Primary Objective

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

6.2. Secondary Objectives

The following secondary objectives will be assessed in subjects with NTDT:

- To evaluate the safety of AG-348
- To determine the effect of AG-348 on markers of hemolysis and erythropoietic activity
- To evaluate the pharmacokinetics of AG-348

6.3. Exploratory Objectives

The exploratory objectives of this study are as follows:

- To determine the effect of AG-348 in subjects with NTDT on the following:
  - Pharmacodynamic (PD) markers of thalassemia
  - Other markers of erythropoietic activity
  - Markers of iron metabolism and indicators of iron overload
  - Markers of oxidative stress and other related markers
  - Transfusion burden
  - Spleen size
- To evaluate the relationship between AG-348 pharmacokinetics and indicators of clinical activity in subjects with NTDT
- To evaluate the relationship between the dose of AG-348 and change in Hb concentrations in subjects with NTDT

6.4. Endpoints

6.4.1. Primary Endpoint

The primary endpoint of this study is the Hb response (HR), defined as a ≥1.0 g/dL increase in Hb concentration from baseline at 1 or more assessments between Week 4 and Week 12 (inclusive). An individual subject's baseline Hb concentration is defined as the average of all of the subject's available Hb concentrations during the Screening Period up to the first dose of study drug.

6.4.2. Secondary Endpoints

The secondary endpoints of this study are as follows:

- The mean change from baseline in Hb concentrations over a continuous 12-week interval from Week 12 to Week 24
- The sustained Hb response (sHR), defined as a subject who has achieved an HR and has achieved a ≥1.0 g/dL increase in Hb concentration at 2 or more evaluable Hb assessments out of the 4 scheduled assessments between the Week 12 Visit and Week 24 Visit (ie, Weeks 12, 16, 20, and 24)
- The delayed Hb response, defined as a subject who has not achieved an HR, but has achieved a ≥1.0 g/dL increase in Hb concentration at 1 or more Hb assessments after Week 12 (ie, Weeks 16, 20, and 24)
- Change from baseline in Hb concentration over the duration of the Extension Period
- Time to first ≥1.0 g/dL increase in Hb concentration
- Change from baseline in markers of hemolysis: reticulocyte count, bilirubin, LDH, and haptoglobin
- Change from baseline in markers of erythropoietic activity: nucleated RBC (NRBC), EPO, and soluble transferrin receptor
- Safety endpoints of this study are as follows:
  - The type, incidence, severity, and relationship to treatment with AG-348 of adverse events (AEs) and serious adverse events (SAEs), AEs of special interest (AESIs), AEs leading to study drug dose reduction, study drug interruption, and study drug discontinuation
  - Changes over time in clinical laboratory tests (serum chemistry, liver function tests [LFTs], LDH, hematology, coagulation, lipids, sex steroids, and urinalysis), physical examination (PE) findings, bone mineral density (BMD) of the hip and lumbar spine, vital signs, and 12-lead electrocardiogram (ECGs) findings
- Pharmacokinetic endpoints of this study are as follows:
  - Pharmacokinetic endpoints include drug concentrations over time and pharmacokinetic parameters of AG-348, including AUC, C_{max}, and others as applicable

6.4.3. Exploratory Endpoints

The exploratory endpoints of this study are as follows:

- Change from baseline in α-, β-, and gamma (γ)-hemoglobin absolute levels and/or ratios
- Change from baseline in other markers of erythropoietic activity: growth differentiation factor (GDF)-15, GDF-11, and erythroferrone
- Change from baseline in markers of iron metabolism and indicators of iron overload
- Change from baseline in markers of oxidative stress: urinary 8-isoprostane, methylmalonic acid, total homocysteine, and other RBC metabolite measurements
- Proportion of subjects requiring transfusions and the total number of RBC units transfused
- Change from baseline in spleen size as assessed by magnetic resonance imaging (MRI)
- Change in Hb concentrations in relation to the dose of AG-348
- Pharmacokinetic/PD endpoints of this study are as follows:
  - Change from baseline in adenosine triphosphate (ATP), 2,3-DPG concentrations, PKR activity, PKR protein levels, and PKR flux assay results
  - Exposure-response (or pharmacokinetic-PD) relationship between relevant pharmacokinetic parameters and endpoints that are indicators of clinical activity

7. STUDY DESIGN

7.1. Study Design Overview

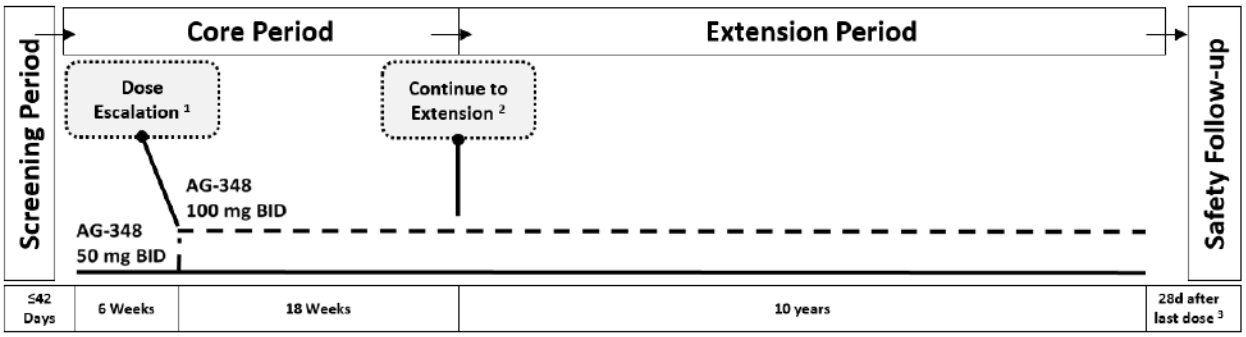
This is a Phase 2, open-label, multicenter study evaluating the efficacy, safety, pharmacokinetics, and PD of treatment with AG-348 in adult subjects with NTDT. This study will consist of a Core Period (up to 24 weeks) followed by an Extension Period (up to 10 years). Approximately 17 subjects with NTDT are planned to be enrolled.

All eligible subjects will receive an initial AG-348 dose of 50 mg BID and may undergo an intrasubject dose escalation to 100 mg BID based on an evaluation of the subject's safety and Hb concentrations as detailed in Section 7.3. Subjects who complete the 24-week Core Period and achieve an HR or delayed HR with an acceptable safety profile may continue AG-348 treatment in the Extension Period after confirmation by the Medical Monitor (or designee) as detailed in Section 7.4.

All subjects who discontinue or interrupt AG-348 should undergo the recommended dose taper regimen (Section 9.3.3), unless an emergency situation justifies discontinuing or interrupting the study drug abruptly. Whether a dose taper is performed or not, subjects discontinuing or interrupting AG-348 should be monitored, as clinically indicated, for signs of hemolysis and worsening of anemia. Subjects will attend the Safety Follow-up Visit 28 days (±4 days) after their last dose of AG-348 (including taper doses).

An overview of the study design is in Figure 4.

Figure 4: Study Schema



Abbreviations: BID = twice daily; d = days.

[1] Subjects may undergo an intrasubject dose escalation at the Week 6 Visit based on an evaluation of safety and Hb concentration (as detailed in Section 7.3) after confirmation by the Medical Monitor (or designee).

[2] Subjects who complete the 24-week Core Period and achieve an HR or a delayed HR with an acceptable safety profile may continue study drug for up to an additional 10 years in the Extension Period, after confirmation by the Medical Monitor (or designee; as detailed in Section 7.4).

[3] The Safety Follow-up Visit will occur 28 days (±4 days) after the subject's last dose of study drug (including taper doses).

7.2. Screening Period

Following signing of the informed consent form (ICF), subjects will enter the 42-day Screening Period to determine eligibility. Screening assessments will be performed as per the Schedule of Assessments (Section 10.1; Table 6).

Eligibility will be confirmed as described in Section 10.3.

7.3. Core Period

Following confirmation of eligibility, subjects will receive AG-348 for a duration of 24 weeks during the Core Period.

All subjects will receive an initial AG-348 dose of 50 mg BID. At the Week 6 Visit, subjects may undergo an intrasubject dose escalation to 100 mg BID based on an evaluation of the subject's safety and Hb concentrations by the treating Investigator. All dose increases must be reviewed and confirmed by the Medical Monitor (or designee).

Subjects will **not** be permitted to increase to 100 mg BID if they meet either of the following criteria:

- Subject has achieved an Hb concentration increase from baseline to 12 g/dL for female subjects or 13 g/dL for male subjects (inclusive) **or**
- Subject has experienced any Grade 3 or greater treatment-emergent adverse events (TEAE) that is considered related to the study drug.

Dose modifications for reasons related to safety may occur at any time during the study. Details for managing such modifications are in Section 9.3.

During the Core Period, all subjects will attend visits on Day 1, then every 2 weeks for the first 8 weeks, and then every 4 weeks thereafter through Week 24.

7.4. Extension Period

Subjects who meet the following criteria will be permitted to continue study drug in the Extension Period after confirmation by the Medical Monitor (or designee):

- Complete the 24-week Core Period
- Achieve an HR (as defined by the primary endpoint, Section 6.4.1) **or** achieve a delayed HR (defined in the secondary endpoints, Section 6.4.2)
- No ongoing Grade 3 or greater TEAE considered related to study drug

During the Extension Period, eligible subjects will continue receiving treatment with AG-348 at the same dose as given at their Week 24 Visit. Study visits will be conducted approximately every 12 weeks for up to 10 years after the subject's Week 24 Visit (as detailed in the Schedule of Assessments, Section 10.1; Table 7). At scheduled telemedicine visits, assessments will be collected from subjects remotely by the Investigator or site staff (as detailed in the Schedule of Assessments, Section 10.1; Table 8) as allowed per local institutional standard of care and regulations; for sites where telemedicine is not permitted by local regulations, subjects are to complete their assessments in person at the site.

7.5. Follow-up and Discontinuation

All subjects who discontinue AG-348 should undergo the recommended dose taper regimen (Section 9.3.3), unless an emergency situation justifies discontinuing the study drug abruptly. Whether a dose taper is performed or not, subjects discontinuing AG-348 should be monitored,

as clinically indicated, for signs of hemolysis and worsening of anemia. Subjects will attend the Safety Follow-up Visit 28 days (±4 days) after their last dose of AG-348 (including taper doses). Subjects with an AE considered related to the study drug will continue to be followed as detailed in Section 11.

7.6. Number of Subjects to be Enrolled

Approximately 17 subjects with NTDT are planned to be enrolled.

7.7. 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 7.7.1) 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 United States 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.

7.7.1. 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 for timing of assessments:

- Alternative distribution of study drug that was intended to be dispensed during scheduled on-site study visits
  - 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 designee), in agreement with the investigator.
  - Secure, trackable delivery methods (delivery service companies [eg, DHL], couriers, and hand delivery) must be used.
  - Sponsor (or designee) approval is required before each shipment. Shipment will be permitted only if, at minimum, a telemedicine visit has been conducted that incorporates appropriate safety assessments.

- Returning unused study drug and empty study drug packaging
  - Return of unused study drug and empty study drug packaging may be delayed until the subject's next visit to the study site. In certain circumstances, the nature of the return process may vary (eg, personal protective equipment may be required).
- Telemedicine visits
  - Telemedicine visits, preferably via video conference, are permissible for all assessments that can be completed via this mode (eg, medical history, concomitant medications, review of AEs).
- Use of laboratories and health care providers not specified in the clinical trial documentation
  - For assessments that cannot be completed via telemedicine, the use of health care providers and laboratories that are not specified in the clinical trial documentation (such as an imaging facility, clinic, or local practice that is more readily accessible by the subject) is permissible for all assessments that can be completed via this mode (eg, blood collection for laboratory assessments, ECG, physical examinations, imaging).
  - Use of a laboratory or health care provider not specified in the clinical trial documentation requires coordination between the subject, the Investigator, and the subject's local health care provider.
  - The Investigator must document their review of the results provided by laboratories and health care providers not specified in the clinical trial documentation.
- Virtual informed reconsent in lieu of in-person informed consent/reconsent
  - 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 to a new mode of completing study procedures). In these instances, consent may be completed virtually and documented in the relevant subject medical records.

7.8. Criteria for Study Closure

This study may be prematurely terminated if, in the opinion of the Sponsor, there is sufficient reasonable cause. In the event of such action, written notification documenting the reason for study termination will be provided to each Investigator.

Circumstances that may warrant study termination include, but are not limited to the following:

- Determination of unexpected, significant, or unacceptable risk to subjects
- Plans to modify, suspend, or discontinue the development of the study drug

- Decisions of competent authorities or Institutional Review Board (IRB)/Independent Ethics Committee (IEC)
- Failure to enroll subjects at an acceptable rate
- Insufficient adherence to protocol requirements
- Other administrative or business reasons

Further details regarding study stopping evaluation for safety reasons are provided in Section 9.3.4 and Section 12.7.3.

Should the study be prematurely terminated, 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 this study:

1. Have provided signed written informed consent prior to performing any study procedure, including screening procedures.
2. Be aged 18 years or older.
3. Have a known medical history of thalassemia, including β-thalassemia intermedia, Hb E β-thalassemia, α-thalassemia (Hb H disease), or β-thalassemia with mutations of 1 or more α genes.
4. Have documented clinical laboratory confirmation of thalassemia by Hb electrophoresis/high-performance liquid chromatography (HPLC) or DNA analysis, either from medical records or during the Screening Period.
5. Have an Hb concentration ≤10.0 g/dL, regardless of sex, based on an average of at least 2 Hb measurements (separated by a minimum of 7 days) during the Screening Period.
6. Be considered non-transfusion-dependent, defined as having no more than 5 units of RBCs transfused during the 24-week period up to the first day of study drug **and** no RBC transfusions in the 8 weeks prior to the first day of study drug.
7. Have adequate organ function, as defined by:
   a. Serum aspartate aminotransferase (AST) ≤2.5 × the upper limit of normal (ULN) and alanine aminotransferase (ALT) ≤2.5 × ULN.
   b. Estimated glomerular filtration rate (GFR) ≥60 mL/min/1.73 m^2, measured GFR ≥60 mL/min, or calculated creatinine clearance (CrCL; Cockcroft-Gault) ≥60 mL/min.
   c. Normal levels of serum bilirubin; or if serum bilirubin >ULN, the elevation must not be associated with choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease. Elevated bilirubin attributed to hemolysis with or without Gilbert's syndrome is not exclusionary.
   d. Absolute neutrophil count (ANC) ≥1.0 × 10^9/L.
   e. Platelet count ≥100 × 10^9/L, in the absence of a spleen, or platelet count ≥50 × 10^9/L, in the presence of a spleen and in the absence of any other cause of thrombocytopenia.
   f. Activated partial thromboplastin time (aPTT) and international normalized ratio (INR) ≤1.25 × ULN, unless the subject is receiving therapeutic anticoagulants.
8. For women of reproductive potential, have a negative serum pregnancy test during the Screening Period and a negative serum or urine pregnancy test on Day 1. Women of reproductive potential are defined as sexually mature women who have not undergone a hysterectomy, bilateral oophorectomy, or tubal occlusion; or who have not been naturally postmenopausal (ie, who have not menstruated at all for at least the preceding 12 months prior to signing informed consent and have an elevated follicle-stimulating hormone (FSH) level indicative of menopause during the Screening Period).
9. For women of reproductive potential as well as men with partners who are women of reproductive potential, be abstinent as part of their usual lifestyle, or agree to use 2 forms

of contraception, 1 of which must be considered highly effective, from the time of giving informed consent, during the study, and for 28 days following the last dose of study drug for women and 90 days following the last dose of study drug for men. A highly effective form of contraception is defined as combined (estrogen and progestin containing) hormonal contraceptives (oral, intravaginal, or transdermal) associated with inhibition of ovulation; progestin-only hormonal contraceptives (oral, injectable, or implantable) associated with inhibition of ovulation; intrauterine device; intrauterine hormone releasing system; bilateral tube occlusion; or vasectomized partner. The second form of contraception can include an acceptable barrier method, which includes male or female condoms with or without spermicide, and cervical cap, diaphragm, or sponge with spermicide. Women of reproductive potential using hormonal contraception as a highly effective form of contraception must also utilize an acceptable barrier method while enrolled in the study and for at least 28 days after their last dose of study drug.

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

8.2. Exclusion Criteria

Subjects who meet any of the following criteria will not be eligible for the study:

1. Have a known history of diagnosis of Hb S or Hb C forms of thalassemia.
2. Have a significant medical condition that confers an unacceptable risk to participating in the study, and/or could confound the interpretation of the study data. Such significant medical conditions include, but are not limited to the following:
   a. Poorly controlled hypertension (defined as systolic blood pressure [BP] >150 mmHg or diastolic BP >90 mmHg) refractory to medical management.
   b. History of recent (within 6 months prior to signing informed consent) congestive heart failure; myocardial infarction or unstable angina pectoris; hemorrhagic, embolic or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.
   c. Iron overload sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac (eg, clinically significant impaired left ventricular ejection fraction), hepatic (eg, cirrhosis or severe fibrosis), or pancreatic (eg, diabetes) dysfunction.
   d. Cardiac dysrhythmias judged as clinically significant by the Investigator.
   e. Heart-rate corrected QT interval-Fridericia's method (QTcF) >450 msec (average of triplicate ECGs) with the exception of subjects with right or left bundle branch block.
   f. Liver disease with histopathological evidence of cirrhosis or severe fibrosis.
   g. Clinically symptomatic cholelithiasis or cholecystitis. Prior cholecystectomy is not exclusionary.
   h. History of drug-induced cholestatic hepatitis.
   i. Have a diagnosis of any other congenital or acquired blood disorder or any other hemolytic process. Allo-immunization will be allowed unless the subject has an ongoing hemolytic process related to antibodies.
   j. Positive test for hepatitis C virus (HCV) antibody (Ab) with evidence of active HCV infection or positive test for hepatitis B surface antigen (HBsAg).
   k. Positive test for human immunodeficiency virus (HIV)-1 or -2Ab.
   l. Active infection requiring the use of parenteral anti-microbial agents or Grade ≥3 in severity (per National Cancer Institute (NCI) Common Terminology Criteria for Adverse events [CTCAE] v4.03) within 1 month prior to the first day of study drug.

m. Diabetes mellitus judged to be under poor control by the Investigator or requiring >3 antidiabetic agents, including insulin (all insulins are considered 1 agent); use of insulin per se is not exclusionary.
n. History of any primary malignancy with the exception of: curatively treated nonmelanomatous skin cancer; curatively treated cervical or breast carcinoma in situ; or other primary tumor treated with curative intent, no known active disease present, and no treatment administered during the last 3 years.
o. Unstable extramedullary hematopoiesis that could pose a risk of imminent neurologic compromise.
p. Current or recent history of psychiatric disorder that, in the opinion of the Investigator or Medical Monitor (or designee), could compromise the ability of the subject to cooperate with study visits and procedures.
q. Pattern or frequency of post-splenectomy sepsis that, in the assessment of the Investigator, could reasonably be expected to interfere with the ability of the subject to complete the study.
r. Lung disease, including pulmonary fibrosis clinical syndrome or pulmonary hypertension requiring oxygen (O_2) therapy.
s. Pulmonary hypertension with tricuspid regurgitation velocity (TRV) ≥3.2 m/s by echo Doppler (obtained within 6 months of Screening).
t. Proteinuria >2 + on dipstick.
u. Clinical diagnosis of osteoporosis (ie, a T-score of −2.5 or lower at the lumbar spine, femur neck, or total hip by bone mineral density testing).
v. Grade ≥3 triglyceride elevations.

3. Have a splenectomy scheduled during the study treatment period or have undergone splenectomy within 12 months prior to signing informed consent.
4. Are currently enrolled in another therapeutic clinical trial involving ongoing therapy with any investigational or marketed product or placebo.
5. Have exposure to any investigational drug, device, or procedure within 3 months prior to the first day of study drug.
6. Have prior exposure to sotatercept (ACE-011), luspatercept (ACE-536), ruxolitinib, or gene therapy.
7. Have a prior bone marrow or stem cell transplant.
8. Are currently pregnant or breastfeeding.
9. Have a history of major surgery within 6 months of signing informed consent. Note that procedures such as laparoscopic gallbladder surgery are not considered major in this context.
10. Are currently receiving medications that are strong inhibitors of cytochrome P450 (CYP)3A4, strong inducers of CYP3A4, strong inhibitors of P-glycoprotein (P-gp), or digoxin (a P-gp sensitive substrate medication) that have not been stopped for a duration of at least 5 days or a timeframe equivalent to 5 half-lives (whichever is longer) prior to the first day of study drug.
11. Are currently receiving chronic anticoagulant therapy, unless started and on a stable dose for at least 28 days prior to first day of study drug.

12. Are currently receiving anabolic steroids, including testosterone preparations, if initiated ≤28 days prior to the first day of study drug.
13. Are currently receiving hematopoietic stimulating agents (eg, erythropoietins, granulocyte colony stimulating factors, thrombopoietins), if initiated ≤8 weeks prior to the first day of study drug.
14. Have a history of allergy to sulfonamides if characterized by acute hemolytic anemia, drug-induced liver injury, anaphylaxis, rash of erythema multiforme type or Stevens-Johnson syndrome, cholestatic hepatitis, or other serious clinical manifestations.
15. Have a history of allergy to AG-348 or its excipients (microcrystalline cellulose, croscarmellose sodium, sodium stearyl fumarate, and mannitol).

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. Subjects may choose to withdraw from the study drug but agree to remain on study for follow-up contact. This decision must be recorded in writing by the study site.

Should a subject decide to withdraw, all efforts will be made to complete and report the protocol-defined study observations as completely as possible and to determine the reason for withdrawal. In the event a subject is withdrawn from study drug or from the study, the Medical Monitor (or designee) must be informed. If there is a medical reason for withdrawal, the subject will remain under the supervision of the Investigator until satisfactory health has returned.

When a subject discontinues study drug or withdraws from the study, the primary reason for discontinuation or withdrawal must be recorded in the appropriate section of the electronic case report form (eCRF) and all efforts will be made to complete and report final study observations as thoroughly as possible.

The Investigator should follow each AE as defined in Section 11.

8.3.1. Withdrawal from Study Drug

Subjects may withdraw or be withdrawn from study drug for any of the following reasons:

- Withdrawal of consent
- Development of an intercurrent medical condition that precludes further participation in the study
- Subject requires use of a prohibited concomitant medication (Section 9.6.1)
- Investigator decision
- Persistent nonadherence to protocol requirements
- Confirmed pregnancy for female subjects only (study therapy should be immediately interrupted based upon a positive urinary human chorionic gonadotropin [hCG] test, and discontinued if confirmed by a serum β-human chorionic gonadotropin [β-hCG] test)

- Lost to follow-up
- AE
- Death
- Study terminated by Sponsor
- Completed

8.3.2. Withdrawal from the Study

The reasons for withdrawal from the study include:

- Withdrawal of consent
- Development of an intercurrent medical condition that precludes further participation in the study
- Subject requires use of a prohibited concomitant medication (Section 9.6.1)
- Investigator decision
- Persistent nonadherence to protocol requirements
- Confirmed pregnancy for female subjects only (study therapy should be immediately interrupted based upon a positive urinary hCG test, and discontinued if confirmed by a serum β-hCG test)
- Lost to follow-up
- AE
- Death
- Study terminated by Sponsor
- Completed

9. STUDY TREATMENT(S)

9.1. Study Drug

Table 1: Investigational Product

Product Name	AG-348
Dosage Form	Tablet
Unit Dose	50 mg
Route of Administration	Oral
Physical Description	Blue film-coated tablet
Manufacturer	Rottendorf Pharma GmbH

9.1.1. Study Drug Packaging and Labeling

AG-348 will be supplied in appropriate containers with child-resistant closures and will be labeled appropriately as investigational medicinal product (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 products must be stored in a secure, limited-access location and may be dispensed only by the Investigator, member of the staff specifically authorized by the Investigator, or party designated to deliver study drug directly to subjects.

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 study drug may be taken with or without food. Subjects will take tablet(s) of the appropriate strength BID, approximately 12 hours apart (ie, 12 hours ± 2 hours). If the dose is not taken 2 hours before or 2 hours after the scheduled dosing time, the dose should be skipped. If a dose is skipped, the next dose should then be taken approximately 24 hours from the previous dose.

Guidance on study drug interruption or discontinuation is provided in Section 9.3.

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. A monitor will review drug accountability at the site on a schedule agreed to by the Sponsor.

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

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

During scheduled on-site study visits, 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 that was intended to be dispensed during scheduled on-site study visits can be shipped directly to a subject, if acceptable by practice and allowed by local regulations. For scheduled telemedicine study visits (see Table 8), study drug will be shipped to the subject by a member of the site staff specifically authorized by the Investigator or by a party designated to deliver study drug directly to subjects, if acceptable by practice and allowed by local regulations. For sites where telemedicine is not permitted by local regulations, subjects are to be dispensed study drug in person at the site.

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

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

9.3. Criteria for Study Drug Reduction, Interruption, and Discontinuation, and Description of Study Stopping Evaluation for Safety Reasons

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

The Investigator will monitor all subjects for safety.

Modification of an individual subject's dose of AG-348 in response to an excessive hemoglobin response will be performed according to Section 9.3.1. Study drug modifications for AEs considered related to study drug will be performed as outlined in Section 9.3.1 (Table 3) except for events of transaminase increases. A separate guide for study drug modifications due to transaminase increases is in Table 4. Transaminase increases are an AESI for AG-348 (details provided in Section 11.2.6).

After careful consideration of the relative risk of withdrawal hemolysis when study drug is discontinued or interrupted abruptly as opposed to when it is reduced gradually, the Investigator should determine if a dose taper (Section 9.3.3) or abrupt discontinuation or interruption of study drug to receive emergency medical treatment is necessary. Whether a dose taper is performed or not, subjects discontinuing or interrupting AG-348 should be monitored, as clinically indicated, for signs of hemolysis and worsening of anemia.

All study drug reductions, interruptions, and discontinuations should be discussed with the Medical Monitor (or designee); all AG-348 dose restarts are to be confirmed by the Medical Monitor (or designee).

9.3.1. Dose Modifications for Excessive Hemoglobin Response

Table 2 will be used to guide dose modifications for subjects with an excessive hemoglobin response. An excessive hemoglobin response is defined as a hemoglobin concentration higher than the concentration that is 2 g/dL (1.24 mmol/L) below the sex-specific ULN.

Table 2: Dose Modifications for Excessive Hemoglobin Response, Study AG348-C-010

Hemoglobin Response[1]	Dose Modification[2]
>(ULN − 2) g/dL and ≤ULN [>(ULN − 1.24) mmol/L and ≤ULN]	Consider decrease to next lowest dose level
>ULN	Decrease to next lowest dose level

Abbreviations: ULN = upper limit of normal, sex-specific

[1] A hemoglobin response >ULN will be reported as an adverse event.

[2] The lowest dose level for dose modifications is 50 mg BID. If the subject is receiving 50 mg BID at the time of the excessive hemoglobin response, the recommended dose taper should be performed to discontinue the subject from study drug.

9.3.2. Study Drug Modifications for Adverse Events

Study drug modifications for related AEs will be performed as outlined in Table 3. Study drug modifications for transaminase increases will be performed as outlined in Table 4.

Table 3: Study Drug Modification for Related Adverse Events (Except Transaminase Increases and Events of Excessive Hemoglobin Response), Study AG348-C-010

Related Adverse Event(s) Severity	Study Drug 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 adverse events (see below).
Grade 3	• First occurrence, perform the recommended dose taper[1] to interrupt the study drug until event resolution to Grade ≤1 or baseline, whichever is lower, within 21 days of suspension, and then restart AG-348 at the next lowest dose level (ie, 100 mg BID to 50 mg BID[2]). • Second occurrence, perform the recommended dose taper[1] to discontinue the study drug.
Grade 4	• Perform the recommended dose taper[1] to discontinue the study drug.

Abbreviations: BID = twice daily.

[1] All subjects who discontinue or interrupt study drug should undergo the recommended dose taper regimen in Section 9.3.3 unless an emergency situation justifies interrupting or discontinuing the study drug abruptly.

[2] If the subject was receiving 50 mg BID at the time of the event, they will not be permitted to restart the study drug; no dose levels under 50 mg BID will be permitted (unless as part of the dose taper).

Table 4: Study Drug Modification for Transaminase Increases, Study AG348-C-010

Transaminase Increase Severity	Study Drug Modification
Grade 1 where result is not >2.5 x baseline	• None required. Follow closely.
Grade 2 or Grade 1 with >2.5 x baseline	• First occurrence, none required but consider performing the recommended dose taper[1] to interrupt the study drug, if deemed necessary by the Investigator and Medical Monitor (or designee). If dose is held, hold until event resolution to Grade ≤1 or baseline, whichever is lower, within 21 days of suspension, and then restart AG-348 at the same dose. • Second occurrence, perform the recommended dose taper[1] to interrupt the study drug until event resolution to Grade ≤1 or baseline, whichever is lower, within 21 days of suspension, and then restart AG-348 at the next lowest dose level (ie, 100 mg BID to 50 mg BID[2]). • Third occurrence, perform the recommended dose taper[1] to discontinue the study drug.
Grade 3	• First occurrence, perform the recommended dose taper[1] to interrupt the study drug until event resolution to Grade ≤1 or baseline, whichever is lower, within 21 days of suspension, and then restart (with confirmation from the Medical Monitor [or designee]) AG-348 at the next lowest dose level (ie, 100 mg BID to 50 mg BID[2]). • Second occurrence, perform the recommended dose taper[1] to discontinue the study drug.
Grade 4	• Perform the recommended dose taper[1] to discontinue the study drug.

Abbreviations: BID = twice daily.

[1] All subjects who discontinue or interrupt study drug should undergo the recommended dose taper regimen in Section 9.3.3 unless an emergency situation justifies interrupting or discontinuing the study drug abruptly.

[2] If the subject was receiving 50 mg BID at the time of the event, they will not be permitted to restart the study drug; no dose levels under 50 mg BID will be permitted (unless as part of the dose taper).

9.3.3. Recommended Dose Taper Regimen

All subjects who discontinue or interrupt study drug should undergo the recommended dose taper regimen in Table 5. This regimen is based on the dose level of study drug being 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 hemolysis and worsening of anemia.

If the recommended dose taper is performed to discontinue study drug, subjects stop taking the study drug after the taper has been completed.

Table 5: Recommended Dose Taper Regimen, Study AG348-C-010

Dose Level of AG-348 (at the time of the dose taper)	First Step ×7 days	Second Step ×7 days
50 mg BID	50 mg QD	n/a
100 mg BID	50 mg BID	50 mg QD

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

9.3.4. Study Stopping Evaluation for Safety Reasons

Safety data will be reviewed on an ongoing basis by the Clinical Study Team throughout the duration of the study (Core and Extension Periods). Upon the occurrence of a drug discontinuation due to an AE, the Agios Safety Management Team will meet to determine the relatedness and seriousness of the event.

Based on the Safety Management Team's cumulative evaluation of treatment-related SAE information, the Bayesian posterior probability will be calculated to determine the chance of having a 20% or greater probability that a subject discontinues from study drug due to a treatment-related SAE. When this posterior probability reaches 85% or higher, it will trigger review by Agios' governing Drug Safety Committee to assess whether the study should be suspended or terminated. Further details on the Bayesian posterior probability trigger and related operating characteristics are provided in Section 12.7.3.

9.4. Duration of Study

9.4.1. Duration of Study Treatment

The maximum duration of study treatment for all subjects will be approximately 10.4 years (542 weeks) including 24 weeks in the Core Period, 516 weeks in the Extension Period (for eligible subjects), and up to 2 weeks for the final dose taper.

9.4.2. Duration of Study Participation

A subject's maximum duration of study participation is approximately 10.5 years (552 weeks) from Screening to the Safety Follow-up Visit.

9.4.3. End of Study

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

9.5. Treatment Compliance

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

9.6. Prior and Concomitant Medications

Prior medications are defined as those administered anytime within the 28 days prior to signing of the ICF until the first day of study drug and concomitant medications are defined as those

administered from the point of first day of study drug through the subject's completion of the study. All prior and concomitant medications must be recorded in the appropriate section of the source documentation and eCRF along with any dosage information, dates of administration, mode of administration, and reason for use. For non-drug therapies, please reference Section 9.7.

9.6.1. Prohibited Medications

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

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

- Strong inhibitors of CYP3A4 (listed in Appendix 5 of the AG-348 IB)
- Products known to inhibit CYP3A4, such as grapefruit or grapefruit juice
- Strong inducers of CYP3A4 (listed in Appendix 5 of the AG-348 IB)
- Hematopoietic stimulating agents (eg, erythropoietins, granulocyte colony stimulating factors, thrombopoietins) must be discontinued no less than 8 weeks prior to the first day of study drug. B12 injections are permitted for subjects with a prior diagnosis of B12 deficiency syndromes. Subjects must be repleted to stability of the Hb and mean corpuscular volume [MCV] prior to enrollment in the study.
- Anabolic steroids, including testosterone preparations, administered for anemia must be discontinued no less than 28 days prior to the first day of study drug.
- Chronic anticoagulant therapy, administered for thrombosis, must be discontinued unless started and on a stable dose for at least 28 days prior to first day of study drug.

As of Protocol Amendment 4, v4.0, the above list of prohibited therapies no longer includes digoxin or strong inhibitors of P-gp as these are no longer prohibited (based on physiologically based pharmacokinetic model simulations that suggested there is no risk of drug-drug interactions between these drugs and AG-348). However no changes were made to the corresponding eligibility criteria (Section 8.2) because enrollment had completed before finalization of Protocol Amendment 3, v4.0.

9.6.2. 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 NTDT, any initiation, completion, or change of iron chelation therapy will be of particular interest. Historical use of chelation therapy up to 1 year before the first day of study drug will be recorded. Data about chelation therapy use during the study will be carefully collected.

AG-348, being a potential CYP3A4 inducer, has the potential to reduce the effectiveness of oral contraceptives. Details regarding contraception use are provided in Inclusion Criterion #8 (Section 8.1).

Appendix 5 of the AG-348 IB includes a comprehensive list of medications that are prohibited or are to be substituted or used with caution.

9.6.3. Allowed Concomitant Medications

Medications other than those specified above (Section 9.6.1) 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 9.6.1.

9.7. Prior and Concomitant Non-Drug Therapies

Prior non-drug therapies determined to be relevant for medical/surgical history and/or eligibility criteria should be recorded. Relevant concomitant non-drug therapies used to treat an AE should be collected from the signing of ICF until the subject completes the study.

Prior transfusion history and on-study transfusion records should be collected as detailed in the Schedule of Assessments, Section 10.1.Prior transfusion history is to be reported for the 24-week period prior to first day of study drug. If available, Hb concentration <7 days before a transfusion should also be collected.

10. ASSESSMENTS

10.1. Schedule of Assessments

The Schedules of Assessments are captured below for the Core Period in Table 6 and for the Extension Period in Table 7 and Table 8.

Table 6: Schedule of Assessments – Core Period, Study AG348-C-010

Visit: [1]	SCRN	D1	W2	W4	W6	W8	W12	W16	W20	W24	Safety FU[2]
Study Day:	**D -42 to D -1**	**1**	**15**	**29**	**43**	**57**	**85**	**113**	**141**	**169**	**28 days after last dose**
Visit Window:		**0**	**±2 D**	**±2 D**	**±2 D**	**±2 D**	**±4 D**	**±7 D**	**±7 D**	**±7 D**	**±4 D**
Procedures:											
Signed informed consent	X										
Confirmation of thalassemia diagnosis[3]	X										
α- and β-globin mutational analysis[4]	X										
Mutational analysis of *UGT1A1* gene promoter[5]		X									
PKLR genotyping[6]		X									
Demographics[7]	X										
Medical/surgical history[8]	X										
Prior medications[9]	X										
Transfusion history[10]	X										
DXA scan	X									X	
Confirmation of eligibility[11]		X									
MRI	X									X	
Physical examination/height and weight[12]	X	X	X	X	X	X	X	X	X	X	X
Vital signs[13]	X	X	X	X	X	X	X	X	X	X	X
12-lead ECG[14]	X	X[15]			X		X[15]			X	
Clinical Laboratory evaluations:											
HBsAg, HCVAb, HIV1 and 2Ab[16]	X										
FSH[17]	X										

Visit: [1]	SCRN	D1	W2	W4	W6	W8	W12	W16	W20	W24	Safety FU[2]
Study Day:	**D -42 to D -1**	**1**	**15**	**29**	**43**	**57**	**85**	**113**	**141**	**169**	**28 days after last dose**
Visit Window:		**0**	**±2 D**	**±2 D**	**±2 D**	**±2 D**	**±4 D**	**±7 D**	**±7 D**	**±7 D**	**±4 D**
Pregnancy test[18]	X	X[18]		X		X	X	X	X	X	X
CBC with differential[19]	XX[38]	X	X	X	X	X	X	X	X	X	X
Haptoglobin		X	X	X	X	X	X	X	X	X	X
Serum chemistry[20]	X	X		X	X		X			X	X
LFTs[21]	X	X	X	X	X	X	X	X	X	X	X
LDH		X	X	X	X	X	X	X	X	X	X
Coagulation studies[22]	X	X			X		X			X	X
Urinalysis[23]	X	X			X		X			X	X
EPO levels		X			X		X			X	
CRP	X	X			X		X			X	
Soluble transferrin receptor		X			X		X			X	
Iron panel[24]	X	X			X		X			X	
Markers of oxidative stress and other related markers[25]		X			X		X			X	
Additional exploratory markers of erythropoietic activity[26]		X			X		X			X	
Lipids[27]	X	X	X	X	X	X	X	X	X	X	X
Sex steroids[28]		X			X	X	X	X	X	X	X
Study drug administration		X									
Trough pharmacokinetic blood sampling[29]			X	X	X	X		X	X	X	
Intensive pharmacokinetic blood sampling[30]		X					X				

Visit: [1]	SCRN	D1	W2	W4	W6	W8	W12	W16	W20	W24	Safety FU[2]
Study Day:	**D -42 to D -1**	**1**	**15**	**29**	**43**	**57**	**85**	**113**	**141**	**169**	**28 days after last dose**
Visit Window:		**0**	**±2 D**	**±2 D**	**±2 D**	**±2 D**	**±4 D**	**±7 D**	**±7 D**	**±7 D**	**±4 D**
Globin chain quantitation (α-, β-, and γ-)		X			X		X			X	
PD Assessments											
ATP, 2,3 DPG[31]		X			X	X	X			X	
PKR protein[32]		X					X			X	
PKR flux assay[32]		X					X				
PKR activity[33]		X					X				
Adverse events[34]	X	X	X	X	X	X	X	X	X	X	X
On-study transfusion record[35]		X	X	X	X	X	X	X	X	X	X
Concomitant medications/procedures		X	X	X	X	X	X	X	X	X	X
Dispense/collect menstrual cycle diary		X		X		X	X	X	X	X	X
Dispense study drug[36]		X	X	X	X	X	X	X	X	X[36]	
Return study drug			X	X	X	X	X	X	X	X	X
Continue to the Extension Period[37]										X	

Abbreviations: 2,3-DPG = 2,3-Diphosphoglycerate; Ab = antibody; ALP = alkaline phosphatase; ALT = alanine aminotransferase; aPTT = activated partial thromboplastin time; AST = aspartate aminotransferase; ATP = adenosine triphosphate; BUN = blood urea nitrogen; CBC = complete blood count; CrCL = creatinine clearance; CRP = C reactive protein; D = day; DXA = dual-energy x-ray absorptiometry; ECG = electrocardiogram; EPO = erythropoietin; FSH = follicle-stimulating hormone; FU = follow-up; GDF = growth differentiation factor; GFR = glomerular filtration rate; Hb = hemoglobin; HBsAg = hepatitis B surface antigen; hCG = human chorionic gonadotropin; HCT = hematocrit; HCV = hepatitis C virus; HDL-C = high-density lipoprotein cholesterol; HIV = human immunodeficiency virus; HPLC = high performance liquid chromatography; ICF = informed consent form; INR = international normalized ratio; 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; MRI = magnetic resonance imaging; NRBC = nucleated red blood cells; NTBI = non-transferrin bound iron; PD = pharmacodynamics; PKR = red blood cell-specific form of pyruvate kinase; RBC = red blood cell; RDW = red cell distribution width; SAE = serious adverse event; SCRN = Screening; TIBC = total iron-binding capacity; *UGT1A1* = uridine diphosphate glucuronosyl transferase 1A1; 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] All assessments will be performed before study drug dosing unless noted otherwise.

[2] Subjects will attend the Safety Follow-up Visit 28 days (±4 days) after their last dose of AG-348 (including taper doses).

[3] Hb electrophoresis/HPLC and/or DNA analysis, either from medical records or during the Screening Period.
[4] α- and β-globin mutational analysis to be conducted by a central laboratory, even if data are available in the subject's medical records. However, eligibility can be based on data from the subject's medical records.
[5] Mutational analysis of *UGT1A1* gene promoter is to be conducted on Day 1, however results are not required prior to dosing on Day 1.
[6] Mutational analysis of *PKLR* genotyping is to be conducted on Day 1, however results are not required prior to dosing on Day 1.
[7] Subject demographic data will include sex, year of birth, race, and ethnicity. Collection of demographic data will be modified by country regulatory requirements, as appropriate. Race and ethnicity will be collected to ensure that any race and ethnicity related specificities in the safety, pharmacokinetics, and/or efficacy of AG-348 can be captured and interpreted accurately.
[8] Medical/surgical history (general and thalassemia-specific) is to be collected, including all relevant prior medical history and current medical conditions.
[9] Prior medications are defined as those administered anytime within the 28 days prior to signing of the ICF until the first day of study drug. Additional data will be recorded regarding the historical use of chelation therapy up to 1 year prior to the first day of study drug.
[10] Transfusion history is to be recorded for the 24-week period prior to first day of study drug. Transfusion history will include dates of transfusions and the number of RBC units transfused. If available, Hb concentration <7 days before a transfusion should also be collected.
[11] A subject will not be considered enrolled (and cannot receive his/her first dose of study drug) until the site has received confirmation of eligibility from the Medical Monitor (or designee).
[12] Complete physical examination (genital and rectal examinations will be performed at the discretion of the Investigator) with weight will be performed. Height to be collected at screening only. For any subjects with leg ulcers, measurements (maximal length and width) are to be obtained at each physical examination.
[13] Vital signs will include systolic and diastolic blood pressure, heart rate, and temperature.
[14] The 12-lead ECGs will be performed after 5 minutes of recumbency and in triplicate.
[15] The ECGs on Day 1 and Week 12 will be performed at 1 hour (±5 min) postdose (prior to pharmacokinetic sample collection).
[16] If a subject tests positive for HCVAb, a test for HCV RNA will be used to determine if the infection is active.
[17] The FSH assessment will be performed only at Screening for applicable female subjects for confirmation of postmenopausal status. Samples should be drawn in the morning (does not need to be fasting).
[18] For applicable female subjects, a serum hCG must be done during Screening and a urine or serum pregnancy test must be done and documented to be negative on Day 1 before administration of the first dose of study drug. A urine or serum pregnancy test must be done monthly and also be done at any point throughout the study if pregnancy is clinically suspected. All pregnancy tests should be performed locally.
[19] CBC with differential will include HCT, Hb, RBC count, absolute reticulocyte count, percent reticulocyte count, MCV, MCH, MCHC, RDW, NRBC, WBC count with differential, and platelet count.
[20] Serum chemistry parameters will include sodium, potassium, chloride, calcium, magnesium, phosphorus, CO_2 or bicarbonate, albumin, total protein, glucose, BUN or urea, creatinine, and uric acid. At Screening, estimated GFR, measured GFR, or calculated CrCL (Cockcroft-Gault) will be assessed.
[21] LFTs will include ALP, ALT, AST, and total, direct, and indirect bilirubin.
[22] Coagulation parameters will include fibrinogen, aPTT, and INR.
[23] Urinalysis will be performed by a dipstick method and will include assessments of protein, glucose, and leukocytes.
[24] The iron panel will assess iron, serum ferritin, TIBC, transferrin saturation, NTBI, and hepcidin.
[25] Markers of oxidative stress and other related markers include urinary 8-isoprostane, methylmalonic acid, and total homocysteine.
[26] The additional exploratory markers of erythropoietic activity will be erythroferrone, GDF-15, and GDF-11.
[27] For lipid testing samples for total cholesterol, LDL-C, HDL-C, and triglyceride, will be collected after an overnight fast.
[28] Sex steroid testing will assess estrone, estradiol, and testosterone (total and free). Samples should be drawn in the morning (does not need to be fasting).
[29] Trough pharmacokinetic samples will be taken predose; within 60 minutes prior to study drug administration
[30] Pharmacokinetic full profile blood sampling will be conducted in all subjects at the Day 1 and Week 12 Visits 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. On days of sample collection, the morning dose of study drug must be administered at the study site. On days where a predose blood sample is required, the study drug must be administered after the predose sample is taken.
[31] ATP, 2,3-DPG and RBC metabolite exploratory measurement will be obtained from the same sample and are to be collected predose. On days where a predose blood sample is required, the study drug must be administered at the study site after the predose sample is taken.

[32] Whole blood samples for PKR flux assay (contingent upon site feasibility) and PKR protein are to be collected predose. On days where a predose blood sample is required, the study drug must be administered at the study site after the predose sample is taken.
[33] Samples for PKR activity assay (contingent upon site feasibility) are to be collected predose and at 2 hours (±5 minutes) postdose on Day 1 and predose only at the Week 12 Visit.
[34] Adverse events will be collected and recorded as detailed in Section 11. Adverse events and SAEs during the Screening Period (following signing of the informed consent form and prior to the first day of study drug) should only be captured if considered study-procedure related. The Investigator should ask the subject for information regarding sleep patterns, and signs and symptoms associated with insomnia.
[35] Transfusion records will be kept while subjects are on study and will include the date and the number of units transfused.
[36] For the Core Period, 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 to a subject's home, if acceptable by practice and allowed by local regulations. Subjects who discontinue or interrupt study drug should undergo the recommended dose taper (Section 9.3.3) unless an emergency situation justifies interrupting or discontinuing study drug abruptly; study drug will be dispensed to these subjects until study drug is completely stopped.
[37] Subjects who are eligible for the Extension Period following confirmation by the Medical Monitor will continue study drug and proceed directly to the Extension Period. Subjects who are eligible for the Extension Period will have a 12-week supply of study drug dispensed at Week 24.
[38] At least 2 Hb measurements are required during the Screening Period (separated by a minimum of 7 days).

Table 7: Schedule of Assessments – Extension Period, Study AG348-C-010 (Week 36 Through Week 120)

Visit:	W36	W48	W60	W72	W84	W96	W108	W120
Visit Window:	±2 W	±2 W	±2 W	±2 W	±2 W	±2 W	±2 W	±2 W
Procedures:								
DXA scan				X				X
MRI				X				X
Physical examination and weight[2]	X	X	X	X	X	X	X	X
Vital signs[3]	X	X	X	X	X	X	X	X
Clinical Laboratory evaluations:								
CBC with differential[4]	X	X	X	X	X	X	X	X
Haptoglobin	X	X	X	X	X	X	X	X
Serum chemistry[5]	X	X	X	X	X	X	X	X
LFTs[6]	X	X	X	X	X	X	X	X
LDH		X		X		X		X
CRP		X		X		X		X
EPO levels		X		X		X		X
Soluble transferrin receptor		X		X		X		X
Iron panel[7]		X		X		X		X
Lipids[8]	X	X	X	X	X	X	X	X
Sex steroids[9]	X	X	X	X	X	X	X	X
Pregnancy test[10]	X	X	X	X	X	X	X	X
Globin chain quantitation (α-, β-, and γ-)		X		X		X		X
Adverse events[11]	X	X	X	X	X	X	X	X
On-study transfusion record[12]	X	X	X	X	X	X	X	X
Concomitant medications/procedures	X	X	X	X	X	X	X	X
Dispense/collect menstrual cycle diary	X	X	X	X	X	X	X	X
Study drug administration	X							

Visit:	W36	W48	W60	W72	W84	W96	W108	W120
Visit Window:	±2 W	±2 W	±2 W	±2 W	±2 W	±2 W	±2 W	±2 W
Dispense study drug[13]	X	X	X	X	X	X	X	X
Return study drug	X	X	X	X	X	X	X	X

Abbreviations: ALP = alkaline phosphatase; ALT = alanine aminotransaminase; AST = aspartate aminotransaminase; BUN = blood urea nitrogen; CBC = complete blood count; CRP = C-reactive protein; D = day; DXA = Dual-energy x-ray absorptiometry; EPO = erythropoietin; FU = follow-up; Hb = hemoglobin; HCT = hematocrit; HDL-C = high-density lipoprotein cholesterol; 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; MRI = magnetic resonance imaging; NRBC = nucleated red blood cell; NTBI = non-transferrin bound iron; RBC = red blood cell; RDW = red cell distribution width; TIBC = total iron-binding capacity; 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, VS, 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 and the study site.

[1] Subjects will attend the Safety Follow-up Visit 28 days (±4 days) after their last dose of AG-348 (including taper doses).

[2] Complete physical examination (genital and rectal examinations will be performed at the discretion of the Investigator) with weight will be performed. For any subjects with leg ulcers, measurements (maximal length and width) are to be obtained at each physical examination.

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

[4] CBC with differential will include HCT, Hb, RBC count, absolute reticulocyte count, percent reticulocyte count, MCV, MCH, MCHC, RDW, NRBC, WBC count with differential, and platelet count.

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

[6] LFTs will include ALP, ALT, AST, and total, direct, and indirect bilirubin.

[7] The iron panel will assess iron, serum ferritin, TIBC, transferrin saturation, NTBI, and hepcidin.

[8] For lipid testing samples for total cholesterol, LDL-C, HDL-C, and triglyceride will be collected after an overnight fast.

[9] Sex steroid testing will assess estrone, estradiol, and testosterone (total and free). Samples should be drawn in the morning (does not need to be fasting).

[10] For applicable female subjects, a urine or serum pregnancy test must be done monthly and also be done at any point throughout the study if pregnancy is clinically suspected. All pregnancy tests should be performed locally 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. At each scheduled visit, Investigators should confirm with the subjects that these tests were performed and confirm the results.

[11] Adverse events will be collected and recorded as detailed in Section 11. The Investigator should ask the subject for information regarding sleep patterns, and signs and symptoms associated with insomnia.

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

[13] Study drug is expected to be dispensed to the subject at the study site during scheduled on-site study visits; under exceptional circumstances and with agreement of the Sponsor (or representative), study drug that was intended to be dispensed during scheduled on-site study visits can be provided to a subject's home, if acceptable by practice and allowed by local regulations. Subjects who discontinue or interrupt study drug should undergo the recommended dose taper (Section 9.3.3), unless an emergency situation justifies interrupting or discontinuing study drug abruptly; study drug will be dispensed to these subjects until study drug is completely stopped.

Table 8: Schedule of Assessments – Extension Period, Study AG348-C-010 (Week 132 Through Week 540 and Follow-up)

Visit:	W132	W144 (Telemedicine[1])	W156 W204 W252 W300 W348 W396 W444 W492	W168 W216 W264 W312 W360 W408 W456 W504 (Telemedicine[1])	W180 W228 W276 W324 W372 W420 W468 W516	W192 W240 W288 W336 W384 W432 W480 W528 (Telemedicine[1])	W540/ EOT	Safety FU[2] 28 days after last dose
Visit Window:	±2 W	±2 W	±2 W	±2 W	±2 W	±2 W	±2 W	±4 D
Procedures:								
DXA scan					X			
MRI					X			
Physical examination and weight[3]	X		X				X	X
Vital signs[4]	X		X		X		X	X
Clinical Laboratory evaluations:								
CBC with differential[5]	X		X		X		X	X
Serum chemistry[6]	X		X		X		X	X
LFTs[7]	X		X		X		X	X
LDH	X		X		X		X	X
Iron panel[8]	X		X		X		X	
Lipids[9]	X		X		X		X	X
Sex steroids[10]	X		X		X		X	X
Pregnancy test[11]	X	X	X	X	X	X	X	X
Adverse events[12]	X	X	X	X	X	X	X	X
On-study transfusion record[13]	X	X	X	X	X	X	X	X
Concomitant medications/procedures	X	X	X	X	X	X	X	X
Dispense/collect menstrual cycle diary	X		X		X		X	X
Study drug administration	X							

Visit:	W132	W144 (Telemedicine[1])	W156 W204 W252 W300 W348 W396 W444 W492	W168 W216 W264 W312 W360 W408 W456 W504 (Telemedicine[1])	W180 W228 W276 W324 W372 W420 W468 W516	W192 W240 W288 W336 W384 W432 W480 W528 (Telemedicine[1])	W540/ EOT	Safety FU[2] 28 days after last dose
Visit Window:	**±2 W**	**±2 W**	**±2 W**	**±2 W**	**±2 W**	**±2 W**	**±2 W**	**±4 D**
Dispense study drug[14]	X	X	X	X	X	X	X	
Return study drug	X		X		X		X	X

Abbreviations: ALP = alkaline phosphatase; ALT = alanine aminotransaminase; AST = aspartate aminotransaminase; BUN = blood urea nitrogen; CBC = complete blood count; CRP = C-reactive protein; D = day; DXA = Dual-energy x-ray absorptiometry; EPO = erythropoietin; FU = follow-up; Hb = hemoglobin; HCT = hematocrit; HDL-C = high-density lipoprotein cholesterol; 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; MRI = magnetic resonance imaging; NRBC = nucleated red blood cell; RBC = red blood cell; RDW = red cell distribution width; TIBC = total iron-binding capacity; 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, VS, 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 and the study site.

[1] Telemedicine visits will be conducted via phone or video call to assess current supply of study drug, remind subjects to complete their menstrual diaries (as applicable), and record adverse events, concomitant medications, transfusions, and pregnancy test results. 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 will attend the Safety Follow-up Visit 28 days (±4 days) after their last dose of AG-348 (including taper doses).

[3] Complete physical examination (genital and rectal examinations will be performed at the discretion of the Investigator) with weight will be performed. For any subjects with leg ulcers, measurements (maximal length and width) are to be obtained at each physical examination.

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

[5] CBC with differential will include HCT, Hb, RBC count, absolute reticulocyte count, percent reticulocyte count, MCV, MCH, MCHC, RDW, NRBC, WBC count with differential, and platelet count.

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

[7] LFTs will include ALP, ALT, AST, and total, direct, and indirect bilirubin.

[8] The iron panel will assess iron, serum ferritin, TIBC, transferrin saturation, and hepcidin.

[9] For lipid testing samples for total cholesterol, LDL-C, HDL-C, and triglyceride will be collected after an overnight fast.

[10] Sex steroid testing will assess estrone, estradiol, and testosterone (total and free). Samples should be drawn in the morning (does not need to be fasting).

[11] For applicable female subjects, a urine or serum pregnancy test must be done monthly and also be done at any point throughout the study if pregnancy is clinically suspected. All pregnancy tests should be performed locally 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. At each scheduled visit, Investigators should confirm with the subjects that these tests were performed and confirm the results.

[12] Adverse events will be collected and recorded as detailed in Section 11. The Investigator should ask the subject for information regarding sleep patterns, and signs and symptoms associated with insomnia.

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

[14] Study drug is expected to be dispensed to the subject at the study site during scheduled on-site study visits; under exceptional circumstances and with agreement of the Sponsor (or representative), study drug that was intended to be dispensed during scheduled on-site study visits can be provided to a subject's home, if acceptable by practice and allowed by local regulations. For scheduled telemedicine study visits, study drug will be shipped directly to the subject if acceptable by practice and allowed by local regulations. For sites where telemedicine is not permitted by local regulations, subjects are to be dispensed study drug in person at the site. Subjects who discontinue or interrupt study drug should undergo the recommended dose taper (Section 9.3.3), unless an emergency situation justifies interrupting or discontinuing study drug abruptly; study drug will be dispensed to these subjects until study drug is completely stopped.

10.2. Screening Assessments

10.2.1. Informed Consent

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

10.2.2. Thalassemia-Specific Assessments for Eligibility

- *Alpha*- and *Beta*- Mutational Analysis: Mutational analysis for α- and β- globin is to be conducted by a central laboratory, even if data are available in the subject's medical records; however, eligibility can be based on data from the subject's medical records.
- Hemoglobin: For eligibility confirmation, at least 2 measurements of Hb must be obtained (separated by a minimum of 7 days)
- Transfusion History: Recorded to confirm NTDT diagnosis for subject eligibility.

10.2.3. General Assessments and Procedures for Eligibility

General assessments during screening to assess eligibility:

- Physical exam, vital signs, and demographics
- Medical, surgical, and medication history

General procedures during screening to assess eligibility:

- Triplicate ECG: only QTcF will be used for determination of eligibility. Details provided in Section 10.5.1.
- Dual-energy X-ray absorptiometry (DXA) and MRI as detailed in Section 10.5.2 and Section 10.4.3, respectively

10.2.4. Laboratory Assessments for Eligibility

Subjects will have the following laboratory assessments during screening:

- HBsAg, HCVAb, and HIV-1 and HIV-2Ab
  - If a subject tests positive for HCVAb, a test for HCV RNA will be used to determine if the infection is active.
- FSH to confirm post-menopausal status (*if applicable per Inclusion Criterion* #8) or serum hCG at Screening and a local urine or serum pregnancy test on Day 1 prior to the first dose of study drug (*if applicable per Inclusion Criterion* #8)
- LFTs, serum chemistry, CBC with differential, and coagulation: to assess organ function and Hb concentration

- Urinalysis: to assess proteinuria
- Lipids: to assess triglycerides

10.3. Confirmation of Eligibility

The Investigator will determine whether each subject meets all of the Inclusion Criteria and none of the Exclusion Criteria (as per Section 8). Eligibility status for key criteria will be confirmed by the Medical Monitor for each subject.

If study ineligibility is due to a transient condition (eg, prohibited concomitant medication, curable medical condition), the subject could be rescreened after the criterion that the subject failed to meet has resolved. If a subject is declared ineligible according to a previous version of the protocol, the subject may be rescreened if the subject could be eligible according to the current version of the protocol.

The following assessments will not need to be repeated at rescreening if they were performed correctly at prior Screening: demographics, medical/surgical history (unless new information needs to be added), MRI unless done more than 6 months prior, DXA scan unless done more than 6 months prior, and *PKLR* genotyping.

10.4. Assessments of Efficacy

The timing of efficacy assessments is provided in the Schedule of Assessments (Section 10.1; Table 6, Table 7, and Table 8). All clinical laboratory evaluations for efficacy are to be performed by the site's local laboratory unless otherwise noted.

10.4.1. Hemoglobin Laboratory Assessment

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

10.4.2. Additional Laboratory Assessments for Efficacy

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

- CBC with differential
- Haptoglobin (central laboratory)
- Bilirubin (LFT panel)
- LDH
- Globin chain quantitation (α-, β-, and γ-) (central laboratory)
- EPO (central laboratory)
- Soluble transferrin receptor (central laboratory)
- Iron panel and related markers (central laboratory)
- Markers of oxidative stress and other related markers (central laboratory)

- Additional exploratory markers of erythropoietic activity (erythroferrone, GDF-15, and GDF-11) (central laboratory)

10.4.3. Magnetic Resonance Imaging

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

10.5. Assessments of Safety

Assessments of safety will include the following:

- Adverse event reporting, as discussed in Section 11
- Concomitant medications and non-drug therapies as discussed in Section 9.6 and Section 9.7, 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

Timing of safety assessments is provided in the Schedule of Assessments (Section 10.1; Table 6, Table 7, and Table 8).

10.5.1. Electrocardiogram

The 12-lead ECGs should be performed following 5 minutes of recumbency and in triplicate. The ECGs will be read promptly by a qualified physician at the study site to detect any eligibility or safety issue. An ECG will be repeated if clinically significant abnormalities are observed, if artifacts are present, or if machine/equipment errors occur.

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 (before first dose for the screening DXA scan) if not technically adequate. The DXA scans will be read and interpreted by the central vendor.

10.5.3. Safety Laboratory Assessments

All clinical laboratory evaluations are to be performed by the site's local laboratory unless otherwise noted below. Blood samples for clinical laboratory evaluations will be collected according to the Schedule of Assessments (Section 10.1; Table 6, Table 7, and Table 8). 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:

- CBC with differential
- Serum Chemistry
- LFTs
- LDH
- Coagulation Studies
- Sex Steroids (central laboratory)
- Fasting Lipids
- Dipstick Urinalysis
- *UGT1A1* Genotyping to detect subjects with Gilbert syndrome (central laboratory)
- *PKLR* Genotyping (central laboratory)

10.5.4. Menstrual Cycle Diary

Menstruating female subjects will be required to fill out a menstrual cycle diary for each menstrual period in order to detect any change in menstrual cycles.

10.6. Pharmacokinetic Assessments

10.6.1. Blood Sample Collection

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

Blood samples for a full pharmacokinetic profile will be collected from all subjects at the Day 1 and Week 12 Visits at multiple time points as specified in the Schedule of Assessments (Section 10.1; Table 6).

Trough predose pharmacokinetic samples will be collected from all subjects as per specifications provided in the Schedule of Assessments (Section 10.1; Table 6).

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.

10.6.2. 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).

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

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

10.7. Pharmacodynamic Assessments

Pharmacodynamic assessments will include measurements in whole blood of levels of the metabolites ATP and 2,3-DPG, measurements of PKR activity in purified red cells, and an assessment in whole blood of glycolytic pathway activity (PKR flux) pre and post-treatment with AG-348. The PKR flux assay (which involves ex-vivo labeling of whole blood with ^{13}C-glucose) and PKR activity assay will only be conducted at clinical sites able to perform these assessments. In addition, whole blood samples will be collected to quantitate PKR protein levels.

On days where a predose blood sample is required, the study drug must be administered after the predose sample is taken.

Red blood cell samples for a PKR activity profile will be collected at the Day 1 Visit at 2 time points as specified in the Schedule of Assessments (Section 10.1; Table 6). Predose sample collection for PKR activity samples will also be collected at the Week 12 Visit.

Trough predose 2,3-DPG, ATP, PKR protein, and PKR flux assay samples will be collected as per specifications provided in the Schedule of Assessments (Section 10.1; Table 6).

Additional analysis of exploratory biomarkers (PKM protein levels and levels of intermediates in the metabolic pathways affected by PKR) to further the understanding of the mechanism of action of AG-348 may be performed on leftover samples collected from subjects who have agreed to this optional analysis in the ICF (Section 10.2.1). Procedures for sample collection and processing will be provided in a separate study laboratory manual.

If sufficient data are obtained, an exposure-response analysis to evaluate the relationship of AG-348 exposure and PD effects with changes in indicators of clinical activity (eg, changes in Hb concentrations) may be performed.

11. ADVERSE EVENTS

11.1. Reporting Period for Adverse Events and Serious Adverse Events

Monitoring of AEs, including frequency, severity, and characterization of SAEs, AESIs, and AEs leading to discontinuation will be conducted throughout the study. Adverse events and SAEs will be recorded in the source documentation and eCRF from the time of the signing of ICF through the subject's completion of study or withdrawal of consent, whichever occurs first.

All AEs should be monitored until resolution of the AE to baseline, the AE is considered stable within the context of the study, the subject is lost to follow-up, or for a period of 28 days from the subject's 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 considered related to study drug that occur ≥28 days post-treatment are to be reported to the Sponsor directly by the Investigator.

Adverse events will be evaluated by the Investigator and recorded 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 NCI CTCAE v4.03 grading system.

11.2. Definition of Adverse Events

11.2.1. Adverse Event

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

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

11.2.2. Serious Adverse Event

An AE or suspected adverse reaction is considered serious if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death
- Life-threatening (meaning that the subject was at immediate risk of death from the reaction as it occurred; but it does not include a reaction that hypothetically might have caused death had it occurred in a more severe form)
- Inpatient hospitalization or prolongation of existing hospitalization. Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered AEs if the illness or disease existed

before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected manner during the study (eg, surgery performed earlier than planned).

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital anomaly/birth defect
- Important medical event. An important medical event is an event that may not result in death, be life-threatening, or require hospitalization, but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in the definitions for SAEs. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

11.2.3. Adverse Events Occurring Secondary to Other Events

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

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

11.2.4. Pre-existing Medical Conditions

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

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

11.2.5. Abnormal Laboratory Values

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

a. Accompanied by clinical symptoms
b. Results in a change in study drug (eg, dose modification, study drug interruption, or study drug discontinuation)
c. Results in a medical intervention (eg, potassium supplementation for hypokalemia) or a change in concomitant therapy
d. Clinically significant in the Investigator’s judgment

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

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

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

11.2.6. Adverse Events of Special Interest

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

11.2.6.1. Transaminase Increase

Transaminase increase is an AESI for AG-348. In the event of a transaminase increase of >2.5 × baseline (defined as the mean of the Screening and Day 1 values) or an increase in AST or ALT to Grade ≥2 in severity, whichever is lower. The study site should report this occurrence to the Sponsor, using the AESI page in the eCRF, within 24 hours of their first knowledge of the event.

An LFT panel should then be performed weekly until the transaminases have decreased to <2.5 × baseline (defined as the mean of the Screening and Day 1 values). Additionally, the following tests should be performed to gain further information on the possible cause of the transaminase increase:

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

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

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

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

The Investigator should ask the subject for information regarding sleep patterns, and 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 Agios 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 sex-specific ULN). All 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 version 4.03. Adverse events not listed by the CTCAE will be graded as follows:

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

11.3.2. Relationship to Study Drug

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

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

11.4. Pregnancy Reporting

Pregnancy is neither an AE nor an SAE, unless a complication relating to the pregnancy occurs (eg, spontaneous abortion, which may qualify as an SAE). However, any pregnancy in a participating female subject or a female sexual partner of a participating male subject that occurs during this study or within 90 days following the last dose of study drug must be reported to the 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 or

female sexual partner of a male subject should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus. Monitoring should continue until conclusion of the pregnancy.

All outcomes of pregnancy 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 must be recorded on the SAE report form (eg, maternal serious complications, spontaneous or therapeutic abortion, ectopic pregnancy, stillbirth, neonatal death, congenital anomaly, or birth defect) and reported within 24 hours in accordance with the procedure for reporting SAEs.

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

12. STATISTICAL METHODS

The study data will be analyzed and reported in the primary clinical study report (CSR) when all subjects have completed the Week 24 Visit or Safety Follow-up Visit (if applicable) or have discontinued the study. A detailed analysis plan for the Core Period efficacy and safety data will be presented in a statistical analysis plan (SAP), which will be finalized before the Core Period database lock.

Once all subjects have completed the Extension Period and Safety Follow-up Visit (as applicable) or have discontinued the study, additional data will be analyzed for long-term safety and efficacy. The analysis will be specified in a SAP addendum or a separate SAP as considered appropriate, which will be finalized before the final database lock.

12.1. Analysis Sets

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

Full Analysis Set (FAS): All subjects who receive at least 1 dose of study drug.

Safety Analysis Set: All subjects who receive at least 1 dose of study drug. In this nonrandomized study, the FAS and the Safety Analysis Set are identical.

12.2. Analysis Periods

There are 2 dosing periods used in the analysis:

- **Core Dosing Period** is from the first dose of study drug in the Core Period to 28 days after the last dose of study drug for subjects that do not enter the Extension Period, or the last of study drug dosed before the Extension Period for subjects who continue to the Extension Period.
- **On-Treatment Dosing Period** is from the first dose of study drug to 28 days after the last dose of study drug in the study.

12.3. Statistical Analysis

12.3.1. General Methods

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

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

Categorical variables will be summarized using counts and percentages.

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

- The individual subject's baseline Hb concentration, hemolysis markers (indirect bilirubin, LDH, haptoglobin), reticulocytes, iron markers, and erythropoietic markers

are each defined as the average of all of the subject's available assessments during the Screening Period through the date of the first dose of study drug.

- For transaminase (AST and ALT) assessments, the baseline will be defined as the average of the Screening Period and Day 1 values.

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

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

12.3.2. Background Characteristics

Subject disposition, demographic and baseline characteristics, prior and 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 FAS unless otherwise specified in the SAP.

12.3.2.1. Subject Disposition

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

- Subjects enrolled
- Subjects enrolled but not dosed
- Subjects enrolled and dosed (FAS)

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

- Completed 24 weeks of study drug
  - By rollover to extension status (Yes vs No)
- Prematurely discontinued study drug in the Core Period and the reasons for study drug discontinuations
- Prematurely discontinued study drug in the Extension Period and the reasons for study drug discontinuations
- Completed study
- Prematurely discontinued the study and the reasons for study discontinuations

12.3.2.2. Demographics and Baseline Characteristics

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

12.3.2.3. Prior and Concomitant Procedures

Prior and concomitant procedures (eg, transfusions) will be summarized based on FAS and included in subjects' listings.

12.3.2.4. Prior and Concomitant Medications

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

- **Prior medication:** any medication that was started before initial dosing of study drug, regardless of when it ended
- **Concomitant medication:** medication continued or newly received during the treatment-emergent period
- **Post-treatment medication:** medication continued or newly received after the treatment-emergent period

A given medication can be classified as a prior medication, a concomitant medication, or a post-treatment medication; both prior and concomitant; both concomitant and post-treatment; or prior, concomitant, and post-treatment. If a medication has a missing or partially missing start/end date or time and it cannot be determined whether it was taken before, during, or after the treatment-emergent period, it will be considered as a prior, concomitant, and post-treatment medication.

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

12.3.3. Study Drug Exposure and Treatment Compliance

12.3.3.1. Study Drug Exposure

Duration of study drug exposure is defined as follows: last dose date – first dose date + 1 day, regardless of any interruptions in dosing. If the last dose date of study drug is missing, the subject's study 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 FAS.

12.3.3.2. Treatment Compliance

Treatment compliance will be assessed by percentage of tablets taken.

Percent of tablets taken will be calculated as follows:

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

Percentage of tablets taken will be summarized descriptively as a quantitative variable (number, mean, SD, median, minimum, and maximum). The number and percentage of subjects whose compliance is <80% or ≥80% will be summarized.

Treatment compliance will be based on the FAS.

12.4. Efficacy Analyses

12.4.1. Analysis for the Primary Endpoint

In the primary analysis, number and percentage of subjects with an HR will be summarized based on FAS, along with the 90% CI based on exact distribution. If the lower bound of the 90% CI is greater than 30%, the null response rate will be rejected. For subjects who discontinue study drug before Week 4, Hb increase at the last available Hb assessment will be imputed (ie, last observation carry forward [LOCF]) and will be used to evaluate the subject's Hb responder status. To evaluate the impact of missing data and LOCF, sensitivity analyses will be conducted to only include subjects who have at least 1 assessment between the Week 4 and the Week 12 Visits (inclusive).

In the analysis of Hb, Hb results within 8 weeks of transfusion will be excluded.

12.4.2. Analyses for the Secondary Endpoints

12.4.2.1. The Mean Change from Baseline in Hemoglobin Concentrations over a Continuous 12-week Interval from Week 12 to Week 24

Average change from baseline in Hb concentrations from Week 12 to Week 24 will be calculated for each subject using all Hb concentrations collected within the Week 12 to Week 24 windows and will be summarized as a continuous variable.

In addition, average change from baseline in Hb concentrations from Week 4 to Week 6, Week 8 to Week 12, Week 16 to Week 24, Week 4 to Week 24, and Week 4 to Week 12 for all subjects in the FAS as well as Hb responders will be summarized descriptively.

12.4.2.2. Sustained Hemoglobin Response

The number and percentage of subjects with sHR will be summarized, along with its 95% CI based on exact distribution. The analysis will be based on FAS. Subjects with ≤1 Hb assessment at Week 12, Week 16, Week 20, and Week 24 will be considered not reaching the sHR. Similar analysis will be repeated including subjects with at least 2 Hb assessments at Weeks 12, 16, 20, and 24.

12.4.2.3. Delayed Hemoglobin Response

The number and percentage of subjects with a delayed Hb response will be summarized, along with its 95% CI based on exact distribution.

12.4.2.4. Time to First ≥1.0 g/dL Increase in Hemoglobin Concentration from Baseline

Days to first ≥1.0 g/dL increase in Hb concentration from baseline will be summarized as a continuous variable as well as in categories (<6, 6 to <12, ≥12 weeks) for Hb responders.

12.4.2.5. Change from Baseline in Hemoglobin Concentration Over the Duration of the Extension Period

In the summary of Hb concentrations and Hb change from baseline at each visit, all available data including those on or after Week 24 visit will be included. However, as only Hb responders

and delayed responders with an acceptable safety profile may continue AG-348 treatment in the Extension Period, data from the visits in the Extension Period are only reflective of these responders and need to be interpreted with caution.

12.4.2.6. Changes in Markers of Hemolysis and Erythropoietic Activity

Changes from baseline in markers of hemolysis and markers of erythropoietic activity at each visit will be summarized and plotted by Hb responder status. Additional modeling may be conducted. Details will be provided in the SAP.

12.5. Safety Analyses

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

- AEs
- Clinical laboratory values
- BMD of the hip and lumbar spine
- Vital signs
- ECG (standard 12-lead) findings
- Physical examination findings

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

12.5.1. Adverse Events

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

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

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

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

- All TEAEs
- TEAEs by relationship
- TEAEs by maximal severity
- TEAEs leading to study drug discontinuation
- TEAEs leading to study drug interruption

- Serious TEAEs
- AESIs
- TEAEs leading to death

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

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

12.5.2. Laboratory Assessments

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

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

Results of urinalysis and the serum and urine pregnancy tests will be listed in individual subject data listings only. In addition, a listing containing individual subject hematology, chemistry, and coagulation values outside the reference ranges will be provided. This listing will include data from scheduled and unscheduled time points.

12.5.3. Bone Mineral Density Findings

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

12.5.4. Vital Signs

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

12.5.5. ECG Findings

The average of ECG triplicates will be used for each scheduled assessment. A summary of raw values and change from baseline values will be provided at each scheduled time point for the following standard digital ECG measurements: PR, QRS, QT, and QTcF, QRS duration, and heart rate.

The number and percentage of subjects will be summarized by maximum 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.

12.5.6. Physical Examination

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

12.6. Pharmacokinetic/Pharmacodynamic Analyses

12.6.1. Pharmacokinetic Analyses

The plasma pharmacokinetic parameters of AG-348 will be computed using non-compartmental methods based on observed plasma concentrations at actual sample collection times. Descriptive statistics (ie, n, mean, 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.

12.6.2. Pharmacodynamic Analyses

Descriptive statistics will be used to summarize PD parameters for the entire population. Pharmacodynamic parameters will be summarized using the following descriptive statistics: n, mean, SD, coefficient of variation, median, minimum and maximum, geometric mean, and geometric coefficient of variation.

If sufficient data are obtained, an exposure-response analysis may be performed to evaluate the relationship of pharmacokinetics of AG-348 with PD parameters and indicators of efficacy or clinical activity. If such an analysis is conducted, it could be described and conducted under a separate analysis plan.

12.7. Interim and Independent Data Monitoring Committee Analyses

12.7.1. Independent Data Monitoring Committee (IDMC) Analysis

No IDMC is planned for this study.

12.7.2. Interim Analyses

No interim analysis is planned for this study.

12.7.3. Statistical Considerations for Study Stopping Evaluation for Safety Reasons

As noted in Section 9.3.4, the study safety will be continuously evaluated by the Agios Safety Management Team. Based on the Safety Management Team's cumulative evaluation of treatment-related SAE information, the Bayesian posterior probability will be calculated to determine the chance of having a 20% or greater probability that a subject discontinues from study drug due to a treatment-related SAE. When this posterior probability reaches 85% or higher, it will trigger Agios' governing Drug Safety Committee to assess whether the study should be suspended or terminated.

Table 9 below shows the Bayesian posterior probability trigger based on a beta prior distribution with α=1 and β=4.

Table 10 shows the operating characteristics of potential suspending or terminating the study based on the Bayesian posterior probability trigger.

Table 9: Bayesian Posterior Probability Trigger With a Maximum of 6 Subjects With Events (Based on a Prior Beta Distribution With α=1 and β=4)

Number of Subjects With an Event of Interest	Stopping Rule Based on Number of Subjects Enrolled
2	≤3
3	≤6
4	≤10
5	≤14
6	≤17

Table 10: Operation Characteristics Based on Simulation Results Using the Bayesian Posterior Probability Trigger

Probability of Having an Event	Probability of the Study Stopping Early
10%	4.4%
15%	12.2%
20%	23.5%
30%	53.9%
40%	80.1%
50%	94.2%

12.8. Determination of Sample Size

With a total of 17 subjects enrolled, the study will have 80% power to reject a 30% null response rate at a one-sided 0.05 type I error rate when the true response rate is 60%.

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 in order to safeguard the rights, safety, and well-being of the subjects. The study will only be conducted at sites where IRB/IEC approval has been obtained. The protocol, IB, ICF, advertisements (if applicable), written information given to the subjects, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB/IEC.

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

13.3. Subject Information and Informed Consent

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

After the study has been fully explained, written informed consent will be obtained from the subject prior to study participation.

The subject's signed and dated informed consent must be obtained before conducting any study-related procedures. The Investigator must maintain the original, signed consent form. A copy of the signed form must be given to the subject.

The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

13.4. Subject Confidentiality

In order to maintain subject privacy, all source documents, study drug accountability records, study reports and communications will identify the subject by the assigned subject number. The

Investigator will grant monitor(s) and auditor(s) from the Sponsor or the Sponsor's designee and regulatory authority(ies) access to the subject's original medical records for verification of data gathered on the source documents and to audit the data collection process. The subject's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

13.5. Protocol Compliance

The Investigator will conduct the study in compliance with the protocol. Modifications to the protocol should not be made without agreement of both the Investigator and the Sponsor. Changes to the protocol will require written IRB/IEC approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to subjects. The IRB/IEC may provide, if applicable, where regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval/favorable opinion of the IRB/IEC. The Sponsor or designee will submit all protocol modifications to the regulatory authority(ies) in accordance with the governing regulations.

When immediate deviation from the protocol is required to eliminate an immediate hazard(s) to subjects, the Investigator will contact the Medical Monitor (or 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

Cappellini MD, Cohen A, Perter J, Taher A, Viprakasit V. *Guidelines for the Management of Transfusion Dependent Thalassemia (TDT).* 3rd ed: Thalassemia International Federation; 2014.

Chakraborty I, Mishra R, Gachhui R, Kar M. Distortion of beta-globin chain of hemoglobin alters the pathway of erythrocytic glucose metabolism through band 3 protein. *Arch Med Res.* 2012;43(2):112-116. doi:10.1016/j.arcmed.2012.02.003

Chen K, Liu J, Heck S, Chasis JA, An X, Mohandas N. Resolving the distinct stages in erythroid differentiation based on dynamic changes in membrane protein expression during erythropoiesis. *Proc Natl Acad Sci U S A.* 2009;106(41):17413-17418. doi:10.1073/pnas.0909296106

Galanello R, Origa R. Beta-thalassemia. *Orphanet J Rare Dis.* 2010;5:11. doi:10.1186/1750-1172-5-11

Grace RF, Layton DM, Galacteros F, et al. Result update from the DRIVE PK study: effects of AG-348, a pyruvate kinase activator, in patients with pyruvate kinase deficiency: updated results from the DRIVE PK study. *Blood.* 2017;130(suppl 1):2194.

Gunn RB, Silvers DN, Rosse WF. Potassium permeability in -thalassemia minor red blood cells. *J Clin Invest.* 1972;51(5):1043-1050. doi:10.1172/JCI106895

Harewood J, Bhimji SS. Alpha thalassemia (hemoglobin H disease). In: *StatPearls.* StatPearls Publishing; 2018.

Karimi M, Cohan N, De Sanctis V, Mallat NS, Taher A. Guidelines for diagnosis and management of Beta-thalassemia intermedia. *Pediatr Hematol Oncol.* 2014;31(7):583-596. doi:10.3109/08880018.2014.937884

Khandros E, Thom CS, D'Souza J, Weiss MJ. Integrated protein quality-control pathways regulate free alpha-globin in murine beta-thalassemia. *Blood.* 2012;119(22):5265-5275. doi:10.1182/blood-2011-12-397729

McColl B, Vadolas J. Animal models of beta-hemoglobinopathies: utility and limitations. *J Blood Med.* 2016;7:263-274. doi:10.2147/JBM.S87955

Musallam KM, Cappellini MD, Wood JC, et al. Elevated liver iron concentration is a marker of increased morbidity in patients with beta thalassemia intermedia. *Haematologica.* 2011;96(11):1605-1612. doi:10.3324/haematol.2011.047852

Musallam KM, Rivella S, Vichinsky E, Rachmilewitz EA. Non-transfusion-dependent thalassemias. *Haematologica.* 2013;98(6):833-844. doi:10.3324/haematol.2012.066845

Musallam KM, Taher AT, Duca L, Cesaretti C, Halawi R, Cappellini MD. Levels of growth differentiation factor-15 are high and correlate with clinical severity in transfusion-independent

patients with beta thalassemia intermedia. *Blood Cells Mol Dis.* 2011;47(4):232-234. doi:10.1016/j.bcmd.2011.07.005

Shaeffer JR. ATP-dependent proteolysis of hemoglobin alpha chains in beta-thalassemic hemolysates is ubiquitin-dependent. *J Biol Chem.* 1988;263(27):13663-13669.

Taher AT, Porter JB, Viprakasit V, et al. Deferasirox effectively reduces iron overload in non-transfusion-dependent thalassemia (NTDT) patients: 1-year extension results from the THALASSA study. *Ann Hematol.* 2013;92(11):1485-1493. doi:10.1007/s00277-013-1808-z

Taher AT, Radwan A, Viprakasit V. When to consider transfusion therapy for patients with non-transfusion-dependent thalassaemia. *Vox Sang.* 2015;108(1):1-10. doi:10.1111/vox.12201

Ting YL, Naccarato S, Qualtieri A, Chidichimo G, Brancati C. In vivo metabolic studies of glucose, ATP and 2,3-DPG in beta-thalassaemia intermedia, heterozygous beta-thalassaemic and normal erythrocytes: 13C and 31P MRS studies. *Br J Haematol.* 1994;88(3):547-554. doi:10.1111/j.1365-2141.1994.tb05072.x

Wiley JS. Increased erythrocyte cation permeability in thalassemia and conditions of marrow stress. *J Clin Invest.* 1981;67(4):917-922. doi:10.1172/jci110140

15. APPENDICES

APPENDIX 1: PROTOCOL AMENDMENT HISTORY

Changes that had a major impact on the conduct of the study are summarized here.

Amendment 3, Version 3.0:

- Removed study visits at Weeks 1, 3, 7, 9, and 10 (Core Period) to reduce burden on the subjects' participation without jeopardizing safety, given current knowledge of the drug's safety profile.
- Adjusted the dose taper regimen to remove the option for a rapid dose taper based upon preliminary data from Study AG348-C-003 (a Phase 2, open-label, randomized, dose-ranging, safety, efficacy, pharmacokinetic, and pharmacodynamic study of AG-348 in adult patients with pyruvate kinase deficiency); a unified dose taper would allow for a streamlined dose reduction.
- Removed the requirement to enroll a minimum of 8 subjects with β-thalassemia: Based on presumed similar mechanism of action for beta and alpha thalassemia, it is no longer considered necessary to place a limit on enrollment of subjects based on the subtype of thalassemia.
- Modified the definition of sustained hemoglobin response to ensure that subjects with a sustained hemoglobin response would be a subset of subjects with a hemoglobin response.
- Added a secondary endpoint for delayed hemoglobin response to create a new endpoint to account for those subjects who do not have a hemoglobin response at or before Week 12 but may have an increase in hemoglobin after Week 12.
- Revised the definition of time to hemoglobin response to include subjects with a delayed hemoglobin response in the analysis.
- Expanded the subject population that is eligible for the Extension Period by including subjects with a delayed hemoglobin response to give Investigators the option to continue study drug for subjects who have demonstrated a delayed hemoglobin response.
- Increased the eligibility threshold for hemoglobin concentration from ≤9 g/dL to ≤10 g/dL to ensure that the study population is reflective of the non−transfusion-dependent thalassemia patient population, which is typically diagnosed because of chronic anemia with hemoglobin concentrations between 7 and 10 g/dL.
- Lengthened the contraception period for male subjects exposed to AG-348 from 28 to 90 days to cover 1 complete spermatogenesis cycle after last exposure to study drug.
- Added instructions for rescreening subjects to allow subjects to be rescreened if study ineligibility was due to a transient condition (eg, prohibited medication).

- Lengthened the reporting period for pregnancies from 28 to 90 days after the last dose of study drug to align with the increase in the length of the contraception period for male subjects from 28 to 90 days.

Amendment 2, Version 2.0:

- Consolidated iron-related secondary and exploratory endpoints into 1 exploratory endpoint for markers of iron metabolism.
- Amended the inclusion criterion for renal function.
- Amended the inclusion criterion for platelet count.
- Redefined hemoglobin (Hb) overshoot, and subsequent study drug dose decrease, to higher than 2 g/dL below the ULN.
- Dose modification guidelines for Grade 3 adverse reactions (considered related to the study drug by the investigator) were made more stringent by indicating that the first occurrence of such an adverse reaction will result in an interruption of study drug with possible restart and a second occurrence will result in discontinuation of study drug.
- Dose modification guidelines for Grade 4 adverse reactions (considered related to the study drug by the investigator) were made more stringent by indicating that the occurrence of such a reaction will result in discontinuation of study drug.
- Added study stopping evaluation for safety reasons.
- Added monthly pregnancy tests for applicable subjects.
- Removed the allowance for local DXA scans performed within 3 months preceding the first day of study drug to be used for the Screening DXA assessment.
- Added further details for assessments to be performed following a transaminase increase.

Amendment 1, Version 1.1 (United Kingdom-specific amendment):

- Dose modification guidelines for Grade 3 adverse reactions (considered related to the study drug by the investigator) were made more stringent by indicating that the first occurrence of such an adverse reaction will result in an interruption of study drug with possible restart and a second occurrence will result in discontinuation of study drug.
- Dose modification guidelines for Grade 4 adverse reactions (considered related to the study drug by the investigator) were made more stringent by indicating that the occurrence of such a reaction will result in discontinuation of study drug.