Open-label Study of PTG-300 in Subjects with Hereditary Hemochromatosis

Protocol Number: PTG-300-06

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Development Phase: Phase 2

Document Version: Protocol Amendment 3, dated 14 September 2020

SPONSOR

Protagonist Therapeutics, Inc

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SIGNATURE PAGE

PROTOCOL AUTHORIZATION

Protocol No.: PTG-300-06

Protocol Title: Open-label Study of PTG-300 in Subjects with Hereditary Hemochromatosis

This study protocol was subjected to critical review. The information it contains is consistent with current knowledge of the risks and benefits of the investigational product, as well as with the moral, ethical, and scientific principles governing clinical research as set out in the current Declaration of Helsinki and the guidelines on Good Clinical Practice.

[redacted]

Chief Development Officer, Clinical Development
Protagonist Therapeutics

[redacted]

Signature

September 17, 2020

Date

INVESTIGATOR'S AGREEMENT

Protocol No.: PTG-300-06

Protocol Title: Open-label Study of PTG-300 in Subjects with Hereditary Hemochromatosis

All documentation for this study that is supplied to me and that has not been previously published will be kept in the strictest confidence. This documentation includes this study protocol, Investigator's Brochure(s) (IB), Case Report Forms (CRFs), and other scientific data.

The study will not be commenced without the prior written approval of a properly constituted Institutional Review Board (IRB). No changes will be made to the study protocol without the prior written approval of the Sponsor and the IRB, except where necessary to avert an immediate hazard to the subjects.

I have read the protocol and agree that the study will be conducted in compliance with the protocol and in accordance with the principles of the current version of the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects), and with 21 CFR Part 50. The conduct of the study will be in accordance with the Integrated Addendum to ICH E6(R1): Guideline for Good Clinical Practice ICH E6(R2) and applicable parts of 21 CFR.

I acknowledge that I am responsible for the overall study conduct. I agree to personally conduct or supervise the described clinical study. I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study at my site are informed about their obligations. Mechanisms are in place to ensure that site staff receive the appropriate information throughout the study.

Investigator Name (Print)	Investigational Site
Investigator Signature	Date

2. SYNOPSIS

Name of Sponsor/Company: Protagonist Therapeutics, Inc.	
Name of Investigational Product: PTG-300	
Protocol Number: PTG-300-06	**Country:** Multiple
Title of Study: Open-label Study of PTG-300 in Subjects with Hereditary Hemochromatosis	
Study center(s): Multicenter	
Phase of development: 2	
Indication: Hereditary hemochromatosis (HH)	
Objectives: 1. To assess the safety and tolerability of PTG-300 in HH subjects. 2. To assess the effect of PTG-300 therapy on transferrin saturation (TSAT) and serum ferritin. 3. To assess the effect of PTG-300 on iron absorption in a subset of subjects. (For subjects participating in the iron absorption substudy). 4. To assess PTG-300's efficacy for treating HH subjects as defined by incidence of phlebotomy. 5. To assess the effect of PTG-300 on quality of life (QoL). 6. To assess the pharmacokinetics (PK), pharmacodynamics (PD), and immunogenicity of PTG-300.	
Study Design: This is a multicenter, single-arm, open-label study in subjects with HH. Subjects will receive subcutaneous PTG-300 for up to 24 weeks. Subjects will start at an initial dose of 10 mg per week subcutaneously. The dose may be increased to 20 mg per week, and subsequently to 40 mg and to 80 mg per week if needed, based on tolerability and the pharmacodynamic marker TSAT. In addition, subcutaneous dosing schedules of 10 mg, 20 mg, and 40 mg twice weekly (Day 1 and either Day 4 or Day 5) may be tested. Subjects' safety and blood iron parameters (serum iron, serum ferritin, transferrin, and TSAT) will be collected to monitor the pharmacodynamic effect of PTG-300. Effect on phlebotomy need and QoL data (36-Item Short Form Health Survey [SF-36], and Patient's Global Impression of Change [PGI-C]) will also be collected and tabulated. A subset of subjects (approximately 6 subjects) will participate in a two-way crossover sequential iron (^{57}Fe) absorption substudy. The oral iron absorption substudy will be conducted once during the 3-week screening period and then following approximately 4 to 6 weeks of PTG-300 treatment.	
Methodology: **Screening (Study Day -21 to -1)** After giving written informed consent, subjects will be screened for eligibility according to inclusion/exclusion criteria (see Diagnosis and Main Criteria for Inclusion section) within 21 days of dosing. Subjects will continue their individual iron restricted diet throughout the study. During this period liver iron concentration (LIC) will be assessed by magnetic resonance imaging (MRI). Subjects will begin treatment with PTG-300 within approximately 7 days following a regular	

phlebotomy, with iron parameters measured before (Screening labs) and after the phlebotomy (pre-dose Study Day 1). Each individual subject's pre-phlebotomy serum ferritin level and TSAT will be used as a criterion for phlebotomy during the study, i.e., the threshold at which a phlebotomy should be considered during the study. The serum ferritin level and TSAT obtained during Screening will be used as baseline to assess the change from these measurements post-dosing with PTG-300.

After eligibility has been confirmed, subjects will be enrolled in the study.

Treatment Period (Week 1 to Week 25)

Study Drug Administration: Dosing with PTG-300 at 10 mg will be initiated in the clinic. On clinic visit days, PTG-300 doses will be administered in the clinic. All doses should be administered in the clinic until the subject or caregiver demonstrates the ability to perform subcutaneous injections properly.

Subjects will begin treatment with 10 mg PTG-300 administered weekly. Subjects will receive their first subcutaneous (SC) injection at the clinic and will be instructed on how to self-administer the injection (or have a caregiver administer the injection) and on appropriate safety monitoring before being discharged.

On Study Day 2, subjects will return to the clinic for serum iron parameters and PTG-300 blood concentration (PK) measurements.

Individual Subject Dose and Schedule Escalation: Individual subject dose and schedule will be determined based on the pharmacodynamic (PD) marker TSAT measured at two time points: once at peak PD effect one day post dose and once at trough PD effect. The intent of dose and schedule adjustment is to reduce TSAT and serum iron levels. The dose of PTG-300 may be increased weekly in a sequential manner from 10 mg to 20 mg and, if necessary, to 40 mg and 80 mg until the TSAT is less than approximately 40% at peak PD effect one day post dose and at the trough PD effect prior to the next dose of PTG-300. In addition, the dose may be adjusted to maintain serum ferritin to no more than 1.5 times the screening value or 150 ng/mL, whichever is greater. Investigators may consider once a week or twice a week dosing of PTG-300 to achieve these objectives. The trough PD effect is the TSAT value measured 7 days after the PTG-300 dose (and before the next dose) for once weekly dosing or 7 days after the first dose in a week for twice weekly dosing. For the twice weekly regimen, the doses should be given at least 3 days apart (e.g., on Day 1 and Day 4 or 5 of each week). The twice weekly PTG-300 dose may be escalated to a maximum of 40 mg twice weekly. To facilitate dose escalation and the identification of a therapeutic dose, investigators may assess TSAT values on days subjects come to the clinic for any dose adjustments. These assessments may occur at times other than the regular scheduled visits indicated on the schedule of events.

Subjects will continue treatment by self-administering PTG-300 at home (or in the clinical center as appropriate) for up to 24 weeks based on acceptable safety and tolerability of the drug (see Safety Monitoring). Subjects will return for clinic visits to have safety assessments; serum iron parameters plus soluble transferrin receptor (sTfR), hepcidin and antidrug antibodies (ADA); and efficacy evaluated according to the schedule of assessments.

Subjects' iron parameters will be monitored throughout the study.

Change in Starting Dose: The starting dose may be increased from 10 mg to 20 mg, 40 mg, or 80 mg as appropriate if <2 of the first 4 subjects (or ≤25% of a larger number of subjects) treated at a given dose have both peak and trough TSAT values approximately <40% and no safety signals have been noted.

Criteria for a Phlebotomy: Subjects should undergo an on-study phlebotomy when the subject's serum ferritin levels and TSAT value are higher than the pre-phlebotomy serum ferritin and TSAT values obtained during Screening. In addition, a phlebotomy may be performed when the Investigator assesses it is necessary for subject care.

Iron Absorption Substudy

Iron absorption subjects will be treated and monitored in an identical manner as the rest of the study population.

Subjects consenting to participate in the iron absorption substudy will receive the stable iron isotope ^{57}Fe orally (approximately 4 mg as ferrous sulfate) on two occasions; once prior to starting PTG-300 (i.e., after the phlebotomy performed during the Screening period; Iron Absorption A), and a second dose approximately 4 to 6 weeks after starting PTG-300 (i.e., post-treatment; Iron Absorption B). The post-treatment Iron Absorption B administration will occur approximately 24 hours following a PTG-300 dose.

Subjects will receive ^{57}Fe orally after an overnight fast of at least 8 hours. Subjects should fast for approximately 4 hours after the ^{57}Fe dose. Blood samples will be collected at 0, 4, 8, 12, and 24 hours for measurement of ^{57}Fe levels and for measurement of iron parameters—serum iron, serum ferritin, transferrin, and TSAT. Additional blood samples will be obtained at 0, 4, 8, 12, and 24 hours following Iron Absorption B for measurement of PTG-300 concentrations.

Post-Treatment / Early Termination Safety Follow-up (Week 29)

Subjects who complete the treatment or who discontinue treatment early will undergo Visit 8/end of treatment (EOT) assessments as well as follow-up safety evaluations approximately 4 weeks after the last dose of study drug (Visit 9/end of study [EOS]).

Safety Monitoring:

Safety will be monitored throughout the study.

Safety measures (including but not limited to physical examinations, clinical laboratory tests, complete blood count [CBC], vital signs, electrocardiograms [ECGs], adverse event monitoring [AEs], and concomitant medications) will be evaluated and recorded over the course of the study.

Any adverse event (AE) that emerges from the time the subject signs an informed consent form (ICF) until Study Exit will be recorded and reported. Safety will be assessed using the National Institutes of Health (NIH) Common Terminology Criteria for Adverse Events (CTCAE) v5.0 criteria.

<u>Safety Criteria for Dose Interval Increase or Dose Reduction</u>

The presence of at least one of the following criteria will require a dose reduction:

- CTCAE Grade 3 hematological toxicity or worsening of ≥2 grades in hematological parameters not clearly resulting from the underlying disease and/or without a clear-cut alternative that does not respond adequately to medical therapy.
- CTCAE Grade 3 non-hematological toxicities not clearly resulting from the underlying disease and/or without a clear-cut alternative that does not respond adequately to medical therapy.
- Treatment-related decrease of hemoglobin (Hgb) from baseline >20% or Grade 2 anemia, whichever is lesser, confirmed by a repeat value and not due to phlebotomy. Prior to resumption of PTG-300 at a reduced dose a follow-up Hgb should be assessed to confirm that Hgb has stabilized or improved to Grade 1.
- Investigator determines that the subject has experienced a clinically significant decrease in Hgb due to PTG-300.

If a treatment-related increase in aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >3 × upper limit of normal (ULN) is seen, dosing with PTG-300 should be held, with resumption at a reduced dose if the AST and/or ALT levels return to baseline within 14 days; if the levels do not return to baseline within 14 days, a magnetic resonance imaging (MRI) should be conducted to assess whether there is an increase in iron accumulation.

Dose reduction has two steps. Initially subjects continue to receive the same dose of PTG-300 but the dosing frequency is reduced from twice weekly to weekly, or from weekly to every 2 weeks. If the subject continues to meet criteria for dose reduction, the dose is then decreased one dose level.

PTG-300 Discontinuation or Stopping

The following are stopping criteria for an individual subject and will result in **discontinuation** of the subject from further treatment with study drug:

- Any CTCAE Grade 4 hematological or non-hematological toxicity not clearly resulting from the underlying disease and/or without a clear-cut alternative.
- CTCAE Grade 3 hematological toxicity or worsening of ≥2 grades in hematological parameters not clearly resulting from the underlying disease and/or without a clear-cut alternative persists over 7 days after adequate PTG-300 dose decrease.
- CTCAE Grade 3 non-hematological toxicities not clearly resulting from the underlying disease and/or without a clear-cut alternative explanation that do not improve within 14 days with maximal medical therapy and adequate PTG-300 dose decrease.
- When the Investigator in consultation with the medical monitor (MM) considers that it is in the subject's best interest to discontinue treatment with PTG-300 (i.e., persistent, worsening or recurrent AEs).
- If a subject requires a dose reduction below 10 mg administered every 2 weeks.
- ALT, AST >5 × ULN and rising.
- ALT, AST remains >5 × ULN with no change in total bilirubin for more than 2 weeks.
- A worsening of clinical symptoms with no other acceptable explanation.
- The product meets Hy's Law
  - Any test showing an increase of serum ALT >3 × ULN or >2 × ULN total bilirubin should be repeated within 48 to 72 hours for ALT, AST, alkaline phosphatase (ALP), and total bilirubin. If the repeat value for ALT or total bilirubin is unchanged or indicates decreasing activity, monitoring should continue at weekly intervals until the results are acceptable or normalized.
- Evidence of increased iron accumulation in liver from baseline.

Number of subjects (planned): Approximately 28 subjects will be enrolled in the study to ensure a total of 20 subjects complete at least 16 weeks of treatment. Approximately 6 subjects will participate in the iron absorption substudy.

Diagnosis and main criteria for inclusion:

Inclusion Criteria: Subjects must meet ALL of the following inclusion criteria to be enrolled:

1. Male and female subjects aged 18 years or older.
2. Have a confirmed diagnosis of HFE-related hereditary hemochromatosis with prior genotype testing.
3. Documented stable phlebotomy for ≥6 months prior to screening; with a phlebotomy frequency of at least 0.25 per month (e.g., received at least three phlebotomies over the previous 12 months, or at least four phlebotomies over the previous 15 months) and a phlebotomy frequency of less than 1 per month.
4. Screening hemoglobin >11.5 g/dL.
5. Serum ferritin <300 ng/mL at screening (before screening phlebotomy).

6. Women of childbearing potential (WOCBP) and men with partners of childbearing potential agree to use a highly effective contraceptive measure (based on the Clinical Trials Facilitation Group [CTFG]) during the duration of the study and for 28 days after the last dose of study drug in the case of women and 90 days after the last dose of study drug in the case of men.
7. Subject understands the study procedures, is willing and able to adhere to study requirements and agrees to participate in the study by giving written informed consent.

Exclusion Criteria: Subjects must meet NONE of the following exclusion criteria to be enrolled:

1. Clinically meaningful laboratory abnormalities at Screening including, but not limited to:
   a. Absolute neutrophil count <1000/µL
   b. Platelet count <100,000/µL
   c. Estimated Glomerular Filtration Rate (eGFR) <40 mL/min/1.73 m^2
   d. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) ≥2.5 × upper limit of normal (ULN) or direct bilirubin >1.5 × ULN
   e. C-reactive protein (CRP) ≥5.0 mg/L.
2. Receiving iron chelation therapy.
3. Receiving erythrocytapheresis.
4. Pregnant or lactating females.
5. Infection requiring hospitalization or intravenous antimicrobial therapy, or opportunistic infection within 3 months of dosing; any infection requiring antimicrobial therapy within 4 weeks of dosing. Prophylactic antibiotics are allowed.
6. Any serious or unstable medical or psychiatric condition that would prevent (as judged by the Investigator) the subject from properly providing informed consent or any condition which would jeopardize compliance with the study or assessment of the study's endpoints.
7. Organ damage from iron overload, that (as judged by the Investigator) may be worsened by participation in this trial.
8. Known primary or secondary immunodeficiency.
9. Known history of autoimmune/inflammatory diseases.
10. Positive for active hepatitis B or hepatitis C or known human immunodeficiency virus (HIV) infection. Active hepatitis B is defined as a known positive hepatitis B surface antigen (HBsAg) result. Active hepatitis C is defined by a positive hep C Ab result and known quantitative hepatitis C virus (HCV) ribonucleic acid (RNA) results greater than the lower level of detection of the assay. Subjects who have a history of HCV infection but have documented sustained virologic response 12 weeks after HCV therapy (SVR12) are eligible.
11. Any surgical procedure requiring general anesthesia within 1 month prior to screening or planned elective surgery during the study (within 3 months for joint replacement surgery).
12. History of invasive malignancies within the last 2 years, except non-melanoma skin cancer and localized cured prostate cancer, cervical cancer, and ductal carcinoma in situ (DCIS).
13. Current or recent history of alcohol dependence or illicit drug use within 6 months prior to screening.
14. Subject is unable to give informed consent.
15. Receipt of an investigational agent within 30 days of screening.

Test product(s), dose, and mode of administration: This study is a single-arm, open-label study.

PTG-300 is formulated in an aqueous buffered solution and pre-filled at 0.5 mL and 1.0 mL into a 1-mL glass syringe. Study drug will be administered by subcutaneous (SC) injections either weekly or twice weekly, using a needle and a pre-filled syringe.

Subjects will receive doses of 10 mg to 80 mg SC weekly or 10 mg to 40 mg SC twice weekly.

^{57}Fe (~4 mg) is provided by Protagonist as a ferrous sulfate solution for oral administration.

Duration of treatment: Individual subjects will participate in this study for up to 31 weeks as follows:

- Screening: Up to 3 weeks
- PTG-300 Treatment: Up to 24 weeks
- Safety Follow-Up: 4 weeks

Study endpoints:

Baseline Definition: The serum ferritin level and TSAT obtained during Screening will be used as baseline.

Efficacy:

- Change from baseline in serum ferritin.
- Duration of time that serum ferritin is below baseline measurement.
- Change from baseline in TSAT.
- Duration of time that TSAT is below baseline measurement.
- Time to first phlebotomy after dosing.
- Change in frequency of phlebotomies based on historical phlebotomy data (24 weeks).
- Proportion of subjects who achieve phlebotomy independence.
- Additional Endpoints:
  - SF-36
  - PGI-C
  - Change in iron absorption following administration of PTG-300. (For subjects completing iron absorption substudy.)
  - PTG-300 pharmacokinetics: PTG-300 concentration as a function of dose and time since last dose.
  - PD: Change from baseline in serum iron, transferrin, and sTfR.
  - Change from baseline in LIC assessed by MRI.
  - Incidence of ADA.

Safety:

- Tabulate frequency of treatment-emergent adverse events (TEAEs) and SAEs; treatment-related TEAEs and SAEs; TEAEs leading to study discontinuation.
- Summarize vital signs, safety laboratory assessments, ECGs, and physical examination findings.

Statistical methods: This trial is exploratory and no rigid criteria for sample size are pre-specified. The choices are based on risk/benefit assessment of accumulating results. However, there will be at least 80% power for a sample size of 20 subjects to detect a 50% reduction in the number of phlebotomies from baseline. The power calculation is based on a hypothesized effect size of 0.5 (mean change = 1, with SD = 2) over 24 weeks using a 1-sided one-sample t-test at alpha = 10% level. Twenty-eight (28) subjects will be enrolled in the study, assuming an approximate 25% dropout.

Summary statistics for all efficacy and PK/PD data will be provided. Frequency distribution for adverse events and summary statistics for laboratory assessments, ECGs and vital signs will be provided.

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

TABLE OF CONTENTS

LIST OF TABLES

4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and specialist terms are used in this study protocol.

Table 1: Abbreviations and Specialist Terms

Abbreviation or Specialist Term	Explanation
ADA	Anti-drug antibody
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
AUC	Area under the curve
BUN	Blood urea nitrogen
CBC	Complete blood count
C_{max}	Maximum concentration
CRA	Clinical research associate
CRF	Case report form
CRP	C-reactive protein
CTCAE	Common Terminology Criteria for Adverse Events
CTFG	Clinical Trials Facilitation Group
DCIS	Ductal carcinoma in situ
DLT	Dose-limiting toxicity
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated glomerular filtration rate
EOS	End of study
EOT	End of treatment
FDA	Food and Drug Administration
FIH	First in human
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HBsAg	Hepatitis B surface antigen
Hct	Hematocrit

Abbreviation or Specialist Term	Explanation
HCV	Hepatitis C virus
Hgb	Hemoglobin
HH	Hereditary hemochromatosis
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human immunodeficiency virus
ICF	Informed consent form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IUD	Intrauterine device
IUS	Intrauterine hormone-releasing system
LIC	Liver iron concentration
MM	Medical monitor
MRI	Magnetic resonance imaging
NIH	National Institutes of Health
NOAEL	no-observed-adverse-effect level
PD	Pharmacodynamic(s)
PE	Physical examination
PI	Principal Investigator
PGI-C	Patient's Global Impression of Change
PK	Pharmacokinetic(s)
PRO	Patient reported outcomes
RBC	Red blood cell
RNA	Ribonucleic acid
QoL	Quality of life
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SAR	Serious adverse reaction
SC	Subcutaneous
SF-36	36-Item Short Form Health Survey
SMC	Safety Monitoring Committee

Abbreviation or Specialist Term	Explanation
sTfR	Soluble transferrin receptor
SVR12	Sustained virologic response 12 weeks after therapy
TEAE	Treatment-emergent adverse event
TSAT	Transferrin saturation
ULN	Upper limit of normal
US	United States
WOCBP	Women of childbearing potential

5. INTRODUCTION

5.1. Background

Protagonist Therapeutics, Inc. (Protagonist) is developing PTG-300, an injectable peptide mimetic of the endogenous hormone hepcidin, as a potential treatment for hereditary hemochromatosis (HH).

HH is a rare genetic disorder that disrupts the body's regulation of iron, causing the body to absorb too much iron from the diet. In HH types I-III, mutations in genes encoding hepcidin regulators, or hepcidin itself lead to diminished production of hepcidin, thus decreasing the inhibitory effect of hepcidin on duodenal iron absorption and causing clinical iron overload (Brissot 2011). Hepcidin deficiency leads to increased circulating transferrin saturation, and ultimately, iron accumulation in organs such as the liver, pancreas, heart, and bone. Iron in excess may induce or favor the development of complications such as cirrhosis, liver cancer, diabetes, heart failure, hypogonadism, but also, complaints such as asthenia and disabling arthritis. If left untreated, HH can lead to morbidity and eventually death (Katsarou 2019). Therapeutic phlebotomy to reduce iron loading is standard treatment for HH. The phlebotomy frequency required for a patient is based on serial measurements of their serum ferritin levels and transferrin saturation (Crownover 2013). Therapeutic phlebotomy is highly efficient in removing excess iron and preventing most of the complications associated with excess iron in the body. However, this treatment does not target the biological mechanisms involved in the iron metabolism disturbance.

In a hepcidin-deficient mouse model of hemochromatosis, a minihepcidin was effective for decreasing iron loading in the liver and heart compared to solvent control in mice that were not iron overloaded (Ramos 2012). These observations suggest that a hepcidin mimetic such as PTG-300 may be effective for preventing iron overload in patients with hemochromatosis.

5.1.1. Rationale for the Development of PTG-300

PTG-300 is a peptidic agent structurally related to natural hepcidin that mimics its inhibitory activity on ferroportin. Hepcidin is a peptide hormone that is the body's main regulator of iron homeostasis (Liu 2016, Ruchala 2014). Hepcidin binds to ferroportin causing internalization and degradation of ferroportin. Hepcidin binding to ferroportin on enterocytes blocks iron absorption from the gastrointestinal tract. The inhibitory effect of hepcidin on ferroportin in macrophages and hepatocytes leads to a decrease in the efflux of iron from these cellular sites of iron storage (Camaschella 2013). Because there are no mechanisms for the efflux of iron from cells other than ferroportin, and because iron is both essential and (in excess) toxic, the role of hepcidin is of central importance to iron regulation and erythropoiesis. Hepcidin's critical role in iron metabolism suggests that hepcidin mimetics such as PTG-300 may be effective for treating a variety of conditions characterized by imbalances in iron metabolism as well as conditions where manipulation of iron availability may alter disease pathogenesis. The rationale for testing PTG-300 in patients with HH is based on its potential ability to restore normal hepcidin function in patients with HH and thereby prevent iron overload.

The overall goal of the current protocol is to evaluate the safety and efficacy of PTG-300 in reducing phlebotomy requirement in patients with HH. The protocol is a Phase 2, single-arm, open-label study (with an iron absorption substudy).

The study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) guidelines, and applicable regulatory requirements.

5.2. Summary of Nonclinical and Clinical Studies

5.2.1. Nonclinical Studies

PTG-300 is a peptidic agent structurally related to natural hepcidin and PTG-300's effects in toxicology studies mimic the effects of hepcidin. In studies of wild type mice, rats and cynomolgus monkeys, single dose subcutaneous or intravenous administration of PTG-300 caused transient decreases in serum iron. The effect on serum iron is brisk in onset (within a few hours of subcutaneous dosing in the thalassemic mouse model) and, depending on the dose, can be quite profound and prolonged. For example, in wild type healthy mice, the effect of a 2.5 mg/kg subcutaneous dose on serum iron persists for at least 30 hours with serum iron declining to a nadir (90% reduction) 10 hours after dosing.

PTG-300 was evaluated in a comprehensive toxicology program including Good Laboratory Practice (GLP)-compliant studies. Specific GLP studies included up to 26-week rat and up to 39-week cynomolgus monkey toxicology studies with 4-week or 5-week reversal periods, as well as genetic toxicology studies to assess potential in vitro mutagenesis, in vitro chromosomal aberrations, and in vivo chromosomal aberrations. Safety pharmacology studies included hERG assay and in vivo studies to assess potential effects on cardiovascular, respiratory, and neurobehavioral parameters.

In toxicology studies, PTG-300 causes exaggerated pharmacological responses in rats and cynomolgus monkeys when dosed for up to 26 or 39 weekly doses. The primary effects of PTG-300 in rats and cynomolgus monkeys are decreases in red blood cell count, hematocrit, and hemoglobin (Hgb) with either compensatory increases of reticulocyte counts at lower doses of PTG-300 or decreases of reticulocyte counts at high doses of PTG-300. Rats dosed with 25 mg/kg PTG-300 for 4 weeks had decreases in red cell mass that were adverse based on magnitude of effect, as well as hepatocellular necrosis that could be secondary to excess iron in liver. Rats dosed with 10 mg/kg/dose for 13 weeks had non-adverse effects consistent with the expected pharmacology of PTG-300. The no-observed-adverse-effect level (NOAEL) was determined to be 10 mg/kg/dose based on a 26-week toxicology study in rats receiving weekly doses of PTG-300 of up to 10 mg/kg/dose. Cynomolgus monkeys dosed with 10 mg/kg PTG-300 for 4 weeks had decreases in red cell mass that were adverse based on magnitude of effect. Cynomolgus monkeys dosed with 6 mg/kg/dose for 13 weeks had no-adverse effects consistent with the expected pharmacology of PTG-300. The NOAEL was determined to be 2 mg/kg/dose following 39 weekly doses of PTG-300 at 0.6, 2, and 6 mg/kg/dose in cynomolgus monkeys. PTG-300 does not cause adverse injection site reactions in rats dosed up to 26 weeks or cynomolgus monkeys dosed up to 39 weeks. In a stand-alone single-dose rat local tolerance study with PTG-300 up to 280 mg/kg (280 mg/mL in dose concentration), the incidence and severity of injection site reactions were generally proportional to the dose and showed incomplete but progressive ongoing reversal by Study Day 14 post-injection. PTG-300 is not mutagenic, and had no apparent effect on cardiovascular, respiratory, or neurobehavioral parameters. All PTG-300-related effects in the GLP toxicology studies were reversed or reversing after a 4- or 5-week treatment-free period. All of the PTG-300-related effects can be monitored clinically.

PTG-300 was also evaluated for embryo-fetal development toxicity in rats and rabbits and fertility and early embryonic development toxicity in both male and female rats. The maternal NOAEL was 3 mg/kg/dose and the NOAEL for the embryo-fetal development was 0.3 mg/kg/dose in the definitive embryo-fetal development toxicity study in rats. The maternal NOAEL was 1 mg/kg/dose and the NOAEL for the embryo-fetal development was 0.3 mg/kg/dose in the definitive embryo-fetal development toxicity study in rabbits. The NOAEL for paternal toxicity and the NOEL for effects on male fertility and early embryonic development were considered to be 10 mg/kg/dose in fertility and early embryonic development toxicity study in male rats. The NOAEL for maternal toxicity and the NOEL for effects on female fertility and early embryonic development were 3 mg/kg/dose in fertility and early embryonic development toxicity study in female rats.

Principle findings in nonclinical toxicology studies are alterations in red cell mass. PTG-300 has no apparent effect on cardiovascular, respiratory, or neurobehavior in safety pharmacology studies. PTG-300 is not genotoxic.

5.2.2. Clinical Studies

To date, PTG-300 has been studied in one completed Phase 1 study in healthy subjects. A Phase 2 study of PTG-300 and the study's open-label extension for treatment of β-thalassemia investigated doses over a range of 3 to 80 mg weekly.

Sixty-three patients have been treated in these Phase 2 studies in β-thalassemia. Combining data for the two studies, subjects have been dosed with PTG-300 for durations ranging from 4 weeks to more than 8 months. More than half of the subjects have been dosed for greater than 16 weeks and more than 750 doses of PTG-300 have been administered. Safety data indicate that PTG-300 is well tolerated with no safety concerns.

The frequency of adverse events did not increase with increasing dose of PTG-300. Most adverse events were typical of those expected for subjects with β-thalassemia. The frequency of adverse events did not increase with increasing dose of PTG-300. Adverse events reported in >5% of subjects were upper respiratory tract infection (11.1%), injection site erythema (9.5%), injection site pain (12.7%), urinary tract infections (7.9%), headache (7.9%), fatigue (6.3%), and pyrexia (6.3%). One subject experienced an SAE of confusion, which was considered by the investigator to be possibly related to PTG-300. The event fully resolved within 2 days. In addition, a Phase 2 study of PTG-300 in patients with polycythemia vera requiring phlebotomy has been initiated.

The completed Phase 1 study was a randomized, double blind, placebo controlled, first-in-human (FIH), single ascending dose (SAD) and repeat dose study of PTG-300 administered subcutaneously (SC) in 62 healthy male subjects to evaluate safety/tolerability, PK and PD activity (as assessed by the effect on serum iron parameters). Subjects received single doses of 1 mg to 80 mg PTG-300 or two 40 mg doses 1 week apart.

Following single dose administration of PTG-300 there was a dose-related reduction in serum iron with a maximal reduction of approximately 65% from baseline. The relationship between dose and effect appeared to plateau at 20 mg with higher doses resulting in a longer duration of effect. This effect was sustained for at least 72 hours at higher doses. In subjects that received two 40-mg doses 1 week apart (QW×2) the effects on iron were comparable for both 40-mg

doses. Serum iron levels did not return to baseline prior to the second dose. Serum iron levels generally returned to baseline approximately 1 to 2 weeks following the second dose of PTG-300. Following single dose administration area under the concentration-time curve (AUC) and maximum concentration (C_{max}) increased with dose; however, the increase was less than dose-proportional.

PTG-300 was well tolerated by subjects in this study, with no SAEs or dose-limiting toxicities (DLTs) at single doses as high as 80 mg and a repeat dose of 40 mg weekly for 2 weeks. The most frequent adverse events (AEs) were injection site reactions (characterized primarily by a transient erythema at the injection site), headaches and upper respiratory tract infections.

These preclinical and clinical data support clinical development of PTG-300 for the treatment of subjects with HH.

5.3. Study Design and Target Population

This is a multicenter, single-arm, open-label study to assess the safety and efficacy of PTG-300 treatment in subjects with HH. Subjects will receive subcutaneous PTG-300 for up to 24 weeks. PTG-300 doses of 10 mg, 20 mg, 40 mg, and 80 mg subcutaneously per week may be tested. In addition, subcutaneous dosing schedules of 10 mg, 20 mg, and 40 mg twice weekly may be tested to achieve a sustained pharmacodynamic effect.

A subset of approximately 6 subjects will participate in a two-way crossover iron absorption substudy.

The study population is subjects with HH who are in maintenance phase and who are on a stable phlebotomy regimen.

5.4. Selection of Doses

Subjects will initially receive a subcutaneous dose of 10 mg per week. The dose was based on data in healthy subjects indicating that a dose of 10 mg PTG-300 results in an average reduction of approximately 19% in serum iron. Iron levels returned to baseline within 1 week.

In the current study a subject's dose may be increased to 20 mg per week and subsequently to 40 mg and 80 mg per week based on insufficient decrease in TSAT and acceptable safety. The PTG-300 doses will be administered as subcutaneous doses once a week or as divided doses twice weekly at least 3 days apart (e.g., on Day 1 and Day 4 or 5 of each week). The intent of dose and schedule adjustment is to reduce TSAT and serum iron levels. These higher doses are based on Phase 1 data that showed greater decreases in serum iron and TSAT and longer duration of decrease compared to the 10 mg dose. A Safety Monitoring Committee (SMC) has determined that doses as high as 80 mg per week administered for 8 weeks or more are safe for treating subjects with β-thalassemia.

5.5. Summary of Potential Risks and Benefits

Based on the experience of PTG-300 dosing in nonclinical studies and a Phase 1 trial in healthy volunteers, the activities of PTG-300 are related to the proposed mechanism of action, allowing clinical monitoring. Immunologic responses to PTG-300 have not been observed in nonclinical species, however administration of PTG-300 carries theoretical liabilities (immunologically

based responses) associated with the systemic administration of a peptide. In the Phase 1 study PTG-300 was well tolerated, with the majority of AEs other than injection site reactions (primarily transient erythema) considered to be unrelated to PTG-300. In two ongoing Phase 2 studies in β-thalassemia patients, PTG-300 has been well tolerated with no safety concerns, and no deaths have been reported by subjects as of a cutoff date of September 1, 2020.

The potential risk in this study is causing anemia in subjects, assuming no iron is available for erythropoiesis.

The nonclinical and clinical data to date suggests that PTG-300 has a good safety profile and that PTG-300 may be effective for decreasing phlebotomy requirements and decreasing symptoms of iron overload. Thus, the potential benefits appear to outweigh the potential risks of PTG-300 and clinical testing of PTG-300 for HH is appropriate.

A subset of approximately 6 subjects will participate in an iron absorption substudy with the stable isotope ^{57}Fe. Subjects will receive an oral administration of approximately 4 mg ^{57}Fe as a ferrous sulfate solution. Stable isotopes are non-radioactive atoms of the same chemical element, which differ only in their number of neutrons (Bodamer 2001). Such studies are common in nutrition science and are often conducted to examine the absorption of selected elements (e.g., iron) from various foods and the impact of various diseases. These tracers are considered safe and may be particularly important for the validation of new treatment modalities, such as novel drug treatments (Bodamer 2001).

6. OBJECTIVES

The study objectives are as follows:

1. To assess the safety and tolerability of PTG-300 in HH subjects.
2. To assess the effect of PTG-300 therapy on transferrin saturation (TSAT) and serum ferritin.
3. To assess the effect of PTG-300 on iron absorption in a subset of subjects. (For subjects participating in the iron absorption substudy).
4. To assess PTG-300's efficacy for treating HH subjects as defined by incidence of phlebotomy.
5. To assess the effect of PTG-300 on quality of life (QoL).
6. To assess the pharmacokinetics (PK), pharmacodynamics (PD), and immunogenicity of PTG-300.

7. INVESTIGATIONAL PLAN

7.1. Overall Study Design

This is a multicenter, single-arm, open-label study in subjects with HH. Subjects will receive subcutaneous PTG-300 for up to 24 weeks. Subjects will start at an initial dose of 10 mg per week subcutaneously. The dose may be increased to 20 mg per week, and subsequently to 40 mg and to 80 mg per week if needed, based on tolerability and the pharmacodynamic marker TSAT. In addition, subcutaneous dosing schedules of 10 mg, 20 mg, and 40 mg twice weekly (Day 1 and either Day 4 or Day 5) may be tested.

Based on a review of the TSAT and safety data from at least the first 4 subjects, the starting dose and/or the dosing regimen may be modified for subsequent subjects to reduce exposure to ineffective doses.

Subjects' safety and blood iron parameters (serum iron, serum ferritin, transferrin, and TSAT) will be collected to monitor the pharmacodynamic effect of PTG-300. Effect on phlebotomy need and quality of life (QoL) data (36-Item Short Form Health Survey [SF-36], and Patient's Global Impression of Change [PGI-C]) will also be collected and tabulated.

A subset of subjects (approximately 6 subjects) will participate in a sequential two-way crossover iron (^{57}Fe) absorption substudy. The oral iron absorption substudy will be conducted once during the 3-week screening period and then following approximately 4 to 6 weeks of PTG-300 treatment.

Subjects will participate in this study for up to 31 weeks as follows:

- Screening: Up to 3 weeks
- PTG-300 Treatment: Up to 24 weeks (may include iron absorption substudy for selected subjects)
- Safety follow up: 4 weeks.

7.1.1. Screening (Study Day -21 to -1)

After giving written informed consent, subjects will be screened for eligibility according to inclusion/exclusion criteria (see Section 8) within 21 days of dosing. Subjects will continue their individual iron restricted diet throughout the study. During this period liver iron concentration (LIC) will be assessed by magnetic resonance imaging (MRI). Subjects will begin treatment with PTG-300 within approximately 7 days following a regular phlebotomy, with iron parameters measured before (Screening labs) and after the phlebotomy (pre-dose Study Day 1). Each individual subject's pre-phlebotomy serum ferritin level and TSAT will be used as a criterion for phlebotomy during the study, i.e., the threshold at which a phlebotomy should be considered during the study. The serum ferritin level and TSAT obtained during Screening will be used as baseline to assess the change from these measurements post-dosing with PTG-300. All concomitant medications received by the subject beginning 30 days prior to Screening should be collected.

After eligibility has been confirmed, subjects will be enrolled in the study. Those subjects who choose to participate and complete informed consent for the iron absorption substudy will complete their Iron Absorption A session during Screening (See Section 7.1.3 below).

7.1.2. Treatment Period (Week 1 to Week 25)

Study Drug Administration: Dosing with PTG-300 at 10 mg will be initiated in the clinic. On clinic visit days, PTG-300 doses will be administered in the clinic. All doses should be administered in the clinic until the subject or caregiver demonstrates the ability to perform subcutaneous injections properly.

Subjects will begin treatment with 10 mg PTG-300 administered weekly. Subjects will receive their first subcutaneous (SC) injection at the clinic and will be instructed on how to self-administer the injection (or have a caregiver administer the injection) and on appropriate safety monitoring before being discharged.

On Study Day 2, subjects will return to the clinic for serum iron parameters and PTG-300 blood concentration (PK) measurements.

Individual Subject Dose and Schedule Escalation: Individual subject dose and schedule will be determined based on the pharmacodynamic (PD) marker TSAT measured at two time points: once at peak PD effect one day post dose and once at trough PD effect. The intent of dose and schedule adjustment is to reduce TSAT and serum iron levels. The dose of PTG-300 may be increased weekly in a sequential manner from 10 mg to 20 mg and, if necessary, to 40 mg and 80 mg until the TSAT is less than approximately 40% at peak PD effect one day post dose and at the trough PD effect prior to the next dose of PTG-300. In addition, the dose may be adjusted to maintain serum ferritin to no more than 1.5 times the screening value or 150 ng/mL, whichever is greater. Investigators may consider once a week or twice a week dosing of PTG-300 to achieve these objectives. The trough PD effect is the TSAT value measured 7 days after the PTG-300 dose (and before the next dose) for once weekly dosing or 7 days after the first dose in a week for twice weekly dosing. For the twice weekly regimen, the doses should be given at least 3 days apart (e.g., on Day 1 and Day 4 or 5 of each week). The twice weekly PTG-300 dose may be escalated to a maximum of 40 mg twice weekly. To facilitate dose escalation and the identification of a therapeutic dose, investigators may assess TSAT values on days subjects come to the clinic for any dose adjustments. These assessments may occur at times other than the regular scheduled visits indicated on the schedule of events.

Subjects will continue treatment by self-administering PTG-300 at home (or in the clinical center as appropriate) for up to 24 weeks based on acceptable safety and tolerability of the drug (Section 7.2). Subjects will return for clinic visits to have safety assessments; serum iron parameters plus soluble transferrin receptor (sTfR), hepcidin and antidrug antibodies (ADA); and efficacy evaluated according to the schedule of assessments.

Subjects' iron parameters will be monitored throughout the study.

Change in Starting Dose: The starting dose may be increased from 10 mg to 20 mg, 40 mg, or 80 mg as appropriate if <2 of the first 4 subjects (or ≤25% of a larger number of subjects) treated at a given dose have both peak and trough TSAT values approximately <40% and no safety signals have been noted.

Criteria for a Phlebotomy: Subjects should undergo an on-study phlebotomy when the subject's serum ferritin levels and TSAT value are higher than the pre-phlebotomy serum ferritin and TSAT values obtained during Screening. In addition, a phlebotomy may be performed when the Investigator assesses it is necessary for subject care.

7.1.3. Iron Absorption Substudy (See Table 3)

Iron absorption subjects will be treated and monitored in an identical manner as the rest of the study population.

Subjects consenting to participate in the iron absorption substudy will receive the stable iron isotope ^{57}Fe orally (approximately 4 mg as ferrous sulfate) on two occasions; once prior to starting PTG-300 (i.e., after the phlebotomy performed during the Screening period, Iron Absorption A), and a second dose after 4 to 6 weeks after starting PTG-300 (i.e., post-treatment; Iron Absorption B). The post-treatment Iron Absorption B administration will occur approximately 24 hours following a PTG-300 dose.

Subjects will receive ^{57}Fe orally after an overnight fast of at least 8 hours. Subjects should fast for approximately 4 hours after the ^{57}Fe dose. Blood samples will be collected at 0, 4, 8, 12, and 24 hours for measurement of ^{57}Fe levels and for measurement of iron parameters—serum iron, serum ferritin, transferrin, and TSAT. Additional blood samples will be obtained at 0, 4, 8, 12, and 24 hours following Iron Absorption B for measurement of PTG-300 concentrations.

Food will be allowed starting 4 hours after the ^{57}Fe administration. Subjects will be monitored in the study center for at least 12 hours, after which subjects may stay overnight or be discharged home (at the discretion of the subject and investigator) and return the next day (approximately 24 hours following ^{57}Fe administration).

7.1.4. Post-Treatment / Early Termination Safety Follow-Up (Week 29)

Subjects who complete the treatment or who discontinue treatment early will undergo Visit 8/end of treatment (EOT) assessments as well as follow-up safety evaluations approximately 4 weeks after the last dose of study drug (Visit 9/end of study [EOS]).

7.2. Safety

Safety will be monitored carefully throughout the study.

Safety measures (including but not limited to physical examinations, clinical laboratory tests, complete blood count [CBC], vital signs, electrocardiograms [ECGs], adverse event monitoring [AEs], and concomitant medications) will be evaluated and recorded over the course of the study according to the schedule of assessments (Table 2). Clinical sites may conduct local laboratory tests if required by site procedures.

Any AE that emerges from the time the subject signs an informed consent form (ICF) until Study Exit will be recorded and reported. Safety will be assessed using National Institutes of Health (NIH) Common Terminology Criteria for Adverse Events (CTCAE) v5.0 criteria. It is recommended that prior to reducing the dose the dosing interval is increased to 2 weeks to alleviate any safety concerns.

7.2.1. Safety Criteria for Dose Interval Increase or Dose Reduction

The presence of at least one of the following criteria will require a dose reduction:

- CTCAE Grade 3 hematological toxicity or worsening of ≥2 grades in hematological parameters not clearly resulting from the underlying disease and/or without a clear-cut alternative that does not respond adequately to medical therapy.
- CTCAE Grade 3 non-hematological toxicities not clearly resulting from the underlying disease and/or without a clear-cut alternative that does not respond adequately to medical therapy.
- Treatment-related decrease of Hgb from baseline >20% or Grade 2 anemia, whichever is lesser, confirmed by a repeat value and not due to phlebotomy. Prior to resumption of PTG-300 at a reduced dose a follow-up Hgb should be assessed to confirm that Hgb has stabilized or improved to Grade 1.
- Investigator determines that the subject has experienced a clinically significant decrease in Hgb due to PTG-300.

If a treatment-related increase in aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >3 × upper limit of normal (ULN) is seen, dosing with PTG-300 should be held, with resumption at a reduced dose if the AST and/or ALT levels return to baseline within 14 days; if the levels do not return to baseline within 14 days, an MRI should be conducted to assess whether there is an increase in iron accumulation.

Dose reduction has two steps. Initially subjects continue to receive the same dose of PTG-300 but the dosing frequency is reduced from twice weekly to weekly, or from weekly to every 2 weeks. If the subject continues to meet criteria for dose reduction, the dose is then decreased one dose level.

7.2.2. PTG-300 Discontinuation or Stopping

The following are stopping criteria for an individual subject and will result in **discontinuation** of the subject from further treatment with study drug:

- Any CTCAE Grade 4 hematological or non-hematological toxicity not clearly resulting from the underlying disease and/or without a clear-cut alternative.
- CTCAE Grade 3 hematological toxicity or worsening of ≥2 grades in hematological parameters not clearly resulting from the underlying disease and/or without a clear-cut alternative persists over 7 days after adequate PTG-300 dose decrease.
- CTCAE Grade 3 non-hematological toxicities not clearly resulting from the underlying disease and/or without a clear-cut alternative explanation that do not improve within 14 days with maximal medical therapy and adequate PTG-300 dose decrease.
- When the Investigator in consultation with the medical monitor (MM) considers that is in the subject's best interest to discontinue treatment with PTG-300 (i.e., persistent, worsening or recurrent AEs).
- If a subject requires a dose reduction below 10 mg administered every 2 weeks.

- ALT, AST >5 × ULN and rising.
- ALT, AST remains >5 × ULN with no change in total bilirubin for more than 2 weeks.
- A worsening of clinical symptoms with no other acceptable explanation.
- The product meets Hy's Law:
  - Any test showing an increase of serum ALT >3 × ULN or >2 × ULN total bilirubin should be repeated within 48 to 72 hours for ALT, AST, alkaline phosphatase (ALP), and total bilirubin. If the repeat value for ALT or total bilirubin is unchanged or indicates decreasing activity, monitoring should continue at weekly intervals until the results are acceptable or normalized.
- Evidence of clinically meaningful increased iron accumulation in liver from baseline.

7.3. Number of Subjects

Approximately 28 subjects will be enrolled in the study to ensure a total of 20 subjects complete at least 16 weeks of treatment. Approximately 6 subjects will participate in the iron absorption substudy.

7.4. Treatment Assignment

This is an open-label study with all subjects receiving PTG-300.

7.5. Dose Adjustment Criteria

The investigator may increase a subject's dose up to 80 mg per week based on lack of pharmacodynamic (TSAT) response and acceptable safety. The starting dose and/or the dosing regimen will be evaluated and modified to enhance clinical benefit (see Section 7.1.2).

Based on pre-defined safety criteria (see Section 7.2.1), a subject's dosing frequency and dose may be reduced.

7.6. Endpoints

Baseline Definition: The serum ferritin level and TSAT obtained during Screening will be used as baseline.

7.6.1. Efficacy

- Change from baseline in serum ferritin.
- Duration of time that serum ferritin is below baseline measurement.
- Change from baseline in TSAT.
- Duration of time that TSAT is below baseline measurement.
- Time to first phlebotomy after dosing.

- Change in frequency of phlebotomies based on historical phlebotomy data (24 weeks).
- Proportion of subjects who achieve phlebotomy independence.
- Additional Endpoints:
  - SF-36
  - PGI-C
  - Change in iron absorption following the administration of PTG-300. (For subjects completing iron absorption substudy.)
  - PTG-300 pharmacokinetics: PTG-300 concentration as a function of dose and time since last dose.
  - PD: Change from baseline in serum iron, transferrin and sTfR.
  - Change from baseline in LIC assessed by MRI.
  - Incidence of ADA.

7.6.2. Safety

Safety is assessed using NIH CTCAE v5.0 criteria.

- Tabulate frequency of treatment-emergent adverse events (TEAEs) and SAEs; treatment-related TEAEs and SAEs; TEAEs leading to study discontinuation.
- Summarize vital signs, safety laboratory assessments, ECGs, and physical examination findings.

8. SELECTION AND WITHDRAWAL OF SUBJECTS

8.1. Subject Inclusion Criteria

Subjects must meet ALL of the following inclusion criteria to be enrolled:

1. Male and female subjects aged 18 years or older.
2. Have a confirmed diagnosis of HFE-related hereditary hemochromatosis with prior genotype testing.
3. Documented stable phlebotomy for ≥6 months prior to screening; with a phlebotomy frequency of at least 0.25 per month (e.g., received at least three phlebotomies over the previous 12 months, or at least four phlebotomies over the previous 15 months) and a phlebotomy frequency of less than 1 per month.
4. Screening hemoglobin >11.5 g/dL.
5. Serum ferritin <300 ng/mL at screening (before screening phlebotomy).
6. Women of childbearing potential (WOCBP) and men with partners of childbearing potential agree to use a highly effective contraceptive measure (based on the Clinical Trials Facilitation Group [CTFG]) during the duration of the study and for 28 days after the last dose of study drug in the case of women and 90 days after the last dose of study drug in the case of men.
7. Subject understands the study procedures, is willing and able to adhere to study requirements and agrees to participate in the study by giving written informed consent.

8.2. Subject Exclusion Criteria

Subjects must meet NONE of the following exclusion criteria to be enrolled.

1. Clinically meaningful laboratory abnormalities at Screening including, but not limited to:
    a. Absolute neutrophil count <1000/µL
    b. Platelet count <100,000/µL
    c. Estimated Glomerular Filtration Rate (eGFR) <40 mL/min/1.73 m^2
    d. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) ≥2.5 × upper limit of normal (ULN) or direct bilirubin >1.5 × ULN
    e. C-reactive protein (CRP) ≥5.0 mg/L.
2. Receiving iron chelation therapy.
3. Receiving erythrocytapheresis.
4. Pregnant or lactating females.
5. Infection requiring hospitalization or intravenous antimicrobial therapy, or opportunistic infection within 3 months of dosing; any infection requiring antimicrobial therapy within 4 weeks of dosing. Prophylactic antibiotics are allowed.
6. Any serious or unstable medical or psychiatric condition that would prevent (as judged by the Investigator) the subject from properly providing informed consent or any

condition which would jeopardize compliance with the study or assessment of the study's endpoints.

7. Organ damage from iron overload, that (as judged by the Investigator) may be worsened by participation in this trial.
8. Known primary or secondary immunodeficiency.
9. Known history of autoimmune/inflammatory diseases.
10. Positive for active hepatitis B or hepatitis C or known human immunodeficiency virus (HIV) infection. Active hepatitis B is defined as a known positive hepatitis B surface antigen (HBsAg) result. Active hepatitis C is defined by a positive hep C Ab result and known quantitative hepatitis C virus (HCV) ribonucleic acid (RNA) results greater than the lower level of detection of the assay. Subjects who have a history of HCV infection but have documented sustained virologic response 12 weeks after HCV therapy (SVR12) are eligible.
11. Any surgical procedure requiring general anesthesia within 1 month prior to screening or planned elective surgery during the study (within 3 months for joint replacement surgery).
12. History of invasive malignancies within the last 2 years, except non-melanoma skin cancer and localized cured prostate cancer, cervical cancer, and ductal carcinoma in situ (DCIS).
13. Current or recent history of alcohol dependence or illicit drug use within 6 months prior to screening.
14. Subject is unable to give informed consent.
15. Receipt of an investigational agent within 30 days of Screening.

8.3. Subject Withdrawal Criteria

Subjects may choose to withdraw at any time.

Subjects should be withdrawn if the Investigator or Sponsor thinks withdrawal is in the subject's best interest.

A subject must be withdrawn if the subject becomes pregnant, is non-compliant with study procedures so that assessment of safety and efficacy are significantly compromised, starts an experimental therapy for HH, or meets dose stopping criteria (Section 7.2.2).

9. STUDY PROCEDURES

Study procedures should be completed as designated in the Schedules of Assessments (Table 2 and Table 3).

Table 2: Schedule of Assessments for Study PTG-300-06

Assessment	Screening[a]	24 Weeks of PTG-300 Treatment									Visit 9/EOS Safety Follow-Up
		Visit 1 (Wk 1)		Visit 2 (Wk 3 [D15])	Visit 3 (Wk 5 [D29])	Visit 4 (Wk 9 [D57])	Visit 5 (Wk 13 [D85])	Visit 6 (Wk 17 [D113])	Visit 7 (Wk 21 [D141])	Visit 8/EOT (Wk 25 [D169])	~4 Wks After Last Dose[c] (~Wk 29)
Study Week[b]	D -21 to -1	D1	D2								
Informed Consent & HIPAA Authorization[d]	X										
Significant medical history	X										
Phlebotomy history (for up to previous 15 months)	X										
Inclusion/exclusion criteria	X										
Demographics	X										
Physical examination (including weight)	X										X
Height	X										
Vital signs	X	X	X		X	X	X	X	X	X	X
12-lead ECG	X	X	X			X		X		X	X
MRI (liver iron concentration)	X									X	
Hepatitis B & C screen	X										
Phlebotomy	X[e]										
Phlebotomy evaluation[f]					X	X	X	X	X	X	
Concomitant medications	X	X			X	X	X	X	X	X	X
Adverse events[g]	X	X	X	X	X	X	X	X	X	X	X
Serum pregnancy test[h]	X										X
Urine pregnancy test[h]		X			X	X	X	X	X	X	
CRP	X					X		X		X	

Assessment	Screening[a]	24 Weeks of PTG-300 Treatment									Visit 9/EOS Safety Follow-Up
		Visit 1 (Wk 1)		Visit 2 (Wk 3 [D15])	Visit 3 (Wk 5 [D29])	Visit 4 (Wk 9 [D57])	Visit 5 (Wk 13 [D85])	Visit 6 (Wk 17 [D113])	Visit 7 (Wk 21 [D141])	Visit 8/EOT (Wk 25 [D169])	~4 Wks After Last Dose[c] (~Wk 29)
Study Week[b]	D -21 to -1	D1	D2								
Iron Absorption Substudy[i]	X				X[i]						
Study drug administration[j]		Weekly									
Clinical laboratory tests[k]	X	X			X[k]	X	X[k]	X	X[k]	X	X
CBC	X	X			X	X	X	X	X	X	X
Iron parameters: Serum iron, transferrin saturation (TSAT), serum ferritin, transferrin[l,m]	X	X	X	X	X	X	X	X	X	X	
Soluble transferrin receptor (sTfR)	X					X				X	
Hepcidin	X					X				X	
PK blood samples for measurement of PTG-300 concentration[m]		X[l]	X	X	X	X	X[l]	X	X	X	X
ADA		X				X		X		X	X
SF-36	X									X	
PGI-C						X		X		X	
Assess study drug compliance					X	X	X	X	X	X	

Abbreviations: ADA = anti-drug antibody, CBC = complete blood count, CRP = C-reactive protein, D = day, ECG = electrocardiogram, EOS = end of study, EOT = end of treatment, HIPAA = Health Insurance Portability and Accountability Act, MRI = magnetic resonance imaging, PGI-C = Patient's Global Impression of Change, SF-36 = 36-Item Short Form Health Survey, sTfR = soluble transferrin receptor, TSAT = transferrin saturation.

NOTE: Subjects may require additional visits one day post dose to assess PD effect for dose escalation.

[a] Subjects may be rescreened if necessary. Rescreening is considered conducting screening tests outside of the 21-day screening window.

[b] Clinical visits should be conducted within an approximately 2-day window (±2 days). All study procedures should occur before study drug administration except as indicated (i.e., PK/PD).

[c] Follow-Up procedures are to occur approximately 4 weeks after last dose whether the subject completes the study or terminates early.
[d] Subjects enrolled at sites in the United States (US) must sign HIPAA authorization prior to the conduct of any study-specific procedures.
[e] Must be conducted within approximately 7 days of PTG-300 dosing. Screening serum iron parameters must be collected before phlebotomy. Note: the pre-phlebotomy serum ferritin level and TSAT will be used as the subject's individual phlebotomy threshold for the study, i.e., the designated level at which a phlebotomy will be considered.
[f] If a subject meets pre-specified criteria for phlebotomy during a visit, schedule a phlebotomy as soon thereafter as possible.
[g] Additional tests or examinations, including physical exams, may be conducted as necessary.
[h] For women of childbearing potential only.
[i] Iron Absorption A is conducted prior to starting PTG-300 (i.e., after the phlebotomy performed during the Screening period). Iron Absorption B should be conducted after approximately 4 to 6 weeks of PTG-300 treatment (one day after PTG-300 administration between Week 4 and Week 6). See Table 3 for the Iron Absorption substudy schedule of events.
[j] Study drug administration: PTG-300 is administered weekly or twice weekly. See Section 7.1.2 for details for monitoring TSAT and dose escalation.
[k] See Appendix 1 for list of clinical laboratory tests. At Visits 3, 5, and 7, tests will include chemistry and CBC panel only (no urinalysis).
[l] These tests should be conducted every 4 weeks at each clinic visit as well as each time point that PTG-300 PK blood samples are collected. In addition, iron parameters must also be measured whenever a subject's dose is escalated (See Section 13.1 and Appendix 2). Additional tests may be obtained as outlined in *Individual Subject Dose and Schedule Escalation* in Section 7.1.2.
[m] All sample collection for iron parameters, PK (PTG-300 concentrations) are conducted pre-dose at each clinic visit, with additional 1-day post-dose collections at Week 1 and following any dose escalation.

Table 3: Schedule of Assessments for Study PTG-300-06 — Iron Absorption Substudy (Parts A and B)

Assessment	Hours Post ^{57}Fe Dosing				
	0	**4**	**8**	**12**	**24**
^{57}Fe administration[a]	X				
Iron parameters: Serum iron, serum ferritin, TSAT, transferrin and ^{57}Fe	X[b]	X	X	X	X
PTG-300 concentration[c]	X[b]	X	X	X	X
Vital signs	X			X	X
Adverse events	X			X	X

Note: Iron Absorption A is conducted prior to starting PTG-300 (i.e., after the phlebotomy performed during the Screening period). Iron Absorption B should be conducted after approximately 4 to 6 weeks of PTG-300 treatment (one day after PTG-300 administration between Week 4 and Week 6).
Abbreviations: TSAT = transferrin saturation.

[a] During Iron Absorption B, ^{57}Fe should be administered approximately one day after PTG-300 administration.

[b] Pre-^{57}Fe dosing.

[c] Only conducted during Iron Absorption B.

10. TREATMENT OF SUBJECTS

10.1. Description of Study Drug

This study is a single-arm, open-label study; there is no placebo or comparator used.

PTG-300 will be provided by Protagonist.

PTG-300 is formulated in an aqueous buffered solution and prefilled at 0.5 mL and 1.0 mL into a 1-mL glass syringe. Study drug will be administered by subcutaneous (SC) injections, using a needle and a pre-filled syringe.

^{57}Fe will be provided by Protagonist as a ferrous sulfate solution for oral administration.

10.2. Concomitant Medications

Subjects must receive appropriate care for HH including phlebotomy. Subjects should receive full supportive care for any other underlying conditions and all medications taken are recorded.

Investigational agents must have been discontinued at least 30 days prior to screening and are not allowed during the study. All concomitant medications received by the subject beginning 30 days prior to Screening should be collected.

10.2.1. Contraceptive Requirements

Women of childbearing potential (WOCBP) and men must use medically acceptable contraception (<1% annual failure rate) during the duration of the study and for 28 days after the last dose of study drug in the case of women and 90 days after the last dose of study drug in the case of men. Medically acceptable methods of contraception that may be used by the subject and/or partner include but are not limited to the following:

- Combined (estrogen and progesterone containing) hormonal contraceptives (for at least 3 months before screening) (oral, intravaginal, or transdermal)
- Progesterone-only hormonal contraception (oral, injectable, or implantable) associated with inhibition of ovulation
- Intrauterine device (IUD)
- Intrauterine hormone-releasing system (IUS)
- Surgically sterile (e.g., vasectomy, hysterectomy, bilateral salpingectomy, or bilateral oophorectomy at least 3 months before dosing)
- Sexual abstinence.

10.3. Treatment Compliance

Study drug accountability and reconciliation will be performed by the study staff and the study monitor(s).

10.4. Randomization and Blinding

This is a single-arm, open-label study; no randomization or blinding is used.

11. STUDY DRUG MATERIALS AND MANAGEMENT

11.1. PTG-300

Specific instructions about the storage, preparation, administration, and accountability of PTG-300 are provided in the Pharmacy Manual.

11.1.1. Study Drug

PTG-300 is provided by Protagonist in prefilled syringes containing PTG-300 in buffered saline solution. PTG-300 is formulated as an aqueous buffered solution, sterilized by filtration, and dispensed into 1 mL glass syringes at a 0.5 mL or 1.0 mL fill volume to deliver doses of 10 mg, 20 mg, and 40 mg. For an 80-mg dose, two 40-mg injections will be administered. Each solution is buffered with sodium acetate and adjusted to pH 5.4 with dilute acetic acid. The tonicity of each solution is adjusted with sodium chloride.

11.1.2. Study Drug Packaging and Labeling

PTG-300 will be provided in labeled syringes with one syringe per box.

11.1.3. Study Drug Storage

PTG-300 Injection must be stored at 2°C to 8°C, protected from light, in a locked area with limited access.

Storage instructions are included on the label. The Pharmacy Manual and Program Guide provide more detailed instructions on drug management.

11.1.4. Study Drug Administration

Injection sites are SC tissue in the abdomen (2 inches away from the navel), triceps, or thighs. Sites should be rotated between injections.

Doses that are administered at the study site on clinic visit days will be given by a trained health care practitioner.

All doses should be administered in the clinic until the subject or caregiver demonstrates the ability to successfully administer PTG-300. Additionally, study drug should be administered in the clinic whenever the subject is scheduled for a dose on a clinic visit day.

11.1.5. Study Drug Accountability

Compliance will be assessment throughout the study.

A record will be maintained by the investigational sites that will account for all dispensing and return of any used and unused study drug.

Investigators will be fully responsible for the security, accessibility, and storage of the study drug while it is at investigational sites. Investigators are also responsible for the education of study staff in the correct administration of the study drug.

11.1.6. Study Drug Handling and Disposal

Upon receipt of the study drug at the investigational site, it must be stored at 2ºC to 8ºC in a secure location with limited access.

At the end of the study the study drug will be reconciled, and a copy of the record given to the study monitor. Upon completion of the study any surplus study drug will either be returned to the Sponsor or be destroyed at the site upon receipt of written approval from Protagonist. Evidence of destruction must be supplied to the study monitor.

Further information of the storage, handling and administration of study drug will be provided in the Pharmacy Manual.

11.2. ^{57}Fe Ferrous Sulfate Solution

^{57}Fe (approximately 4 mg) is provided by Protagonist as an oral ferrous sulfate solution.

Further information of the storage, handling, and administration of ^{57}Fe will be provided in the Pharmacy Manual.

12. ASSESSMENT OF EFFICACY

12.1. Clinical Endpoints

12.1.1. Phlebotomy and Iron Parameters

Intermittent therapeutic phlebotomy is standard of care for patients with HH. During the study the timing of a subject's phlebotomy will be based on their established TSAT and serum ferritin pre-phlebotomy threshold.

During the screening phase, subjects will have a phlebotomy within approximately 7 days prior to PTG-300 dosing. Each individual subject's pre-phlebotomy serum ferritin level will be used as their individual phlebotomy threshold, i.e., the designated level at which a phlebotomy may be performed in the study.

Subjects' iron parameters will be monitored throughout the study. A subject will receive a therapeutic phlebotomy during the study if his/her TSAT and serum ferritin is above their pre-phlebotomy level at Screening.

12.1.2. Iron Absorption (Iron Absorption Substudy)

Subjects consenting to participate in the iron absorption substudy will have their iron absorption measured at two time points, pre-treatment with PTG-300 (Iron Absorption A) and after approximately 4 to 6 weeks of treatment with PTG-300 (Iron Absorption B). The purpose of the iron absorption substudy is to quantify the absorption of iron, marked by the ^{57}Fe isotope and the effect of PTG-300 on absorption of iron following oral dosing.

12.2. Quality of Life Instruments

12.2.1. Medical Outcome Study Questionnaire Short Form Health Survey (SF-36)

The Medical Outcome Study Questionnaire Short Form Health Survey (SF-36; Appendix 3) is a 36-item short-form (SF-36) questionnaire constructed to survey health status in the Medical Outcomes Study (MOS) (Ware 1992). The SF-36 includes one multi-item scale that assesses eight health concepts: 1) limitations in physical activities because of health problems; 2) limitations in social activities because of physical or emotional problems; 3) limitations in usual role activities because of physical health problems; 4) bodily pain; 5) general mental health (psychological distress and well-being); 6) limitations in usual role activities because of emotional problems; 7) vitality (energy and fatigue); and 8) general health perceptions.

12.2.2. Patient Global Impression of Change (PGI-C)

Patient Global Impression of Change (PGI-C; Appendix 4) is a self-report measure that reflects a patient's belief about the efficacy of treatment. The patient will compare his/her condition from the start of the study on a 7-point scale ranging from "Very much worse" (1) to "Very much improved" (7) at the time of the assessment.

12.3. Anti-Drug Antibodies (ADA)

As PTG-300 is a protein drug, subjects may mount an immune response to PTG-300 producing ADA. Development of ADA will be monitored throughout the study. Samples will be obtained pre-dose at the time points specified in Table 2.

Should any subject develop ADA, an assessment will be made whether these are neutralizing and the impact of ADA on PTG-300's safety and efficacy in that subject will be evaluated.

13. PHARMACOKINETIC, PHARMACODYNAMIC AND IRON ABSORPTION ASSESSMENTS

Trough concentrations of PTG-300 by dose will be assessed. In addition, any additional pharmacokinetic parameters that can be estimated will be reported.

13.1. Blood Sample Collection Schedule in Each Study Period

13.1.1. Sample Collection for All Subjects

Blood draws to assess concentration of PTG-300 will be collected during each clinic visit prior to a dose of PTG-300 being administered and one day after PTG-300 dose escalation.

Blood draws to assess iron parameters will be collected during each clinic visit pre-dose PTG-300, and one day after each PTG-300 dose escalation.

See Schedule of Assessments, Table 2.

13.1.2. Sample Collection for Iron Absorption Substudy

Blood samples (5 mL) for measurement of ^{57}Fe, will be collected at the following time points: 0 (pre-dose ^{57}Fe) and at 4, 8, 12, and 24 hours post-dose ^{57}Fe (within 10% nominal time).

Blood samples (5 mL) for measurement of iron parameters will be collected pre-dose ^{57}Fe and at 4, 8, 12, and 24 hours post-dose ^{57}Fe (within10% nominal time).

Blood samples (5 mL) for measurement of concentrations of PTG-300 will be obtained at the following time points during Iron Absorption B: at pre-dose ^{57}Fe, and at 4, 8, 12, and 24 hours post-dose ^{57}Fe (within 10% nominal time).

See Schedule of Assessments for Iron Absorption substudy, Table 3.

13.2. Samples Per Subject

Approximately 11 blood samples for PK and iron parameters (approximately 5 mL each) will be collected if a subject completes the entire study. In addition, blood samples will be taken for assessment of hematology and clinical chemistry. A total of approximately 160 mL of blood will be drawn over the 31-week study period for subjects who complete the study. Subjects that require dose escalation to 80 mg may have up to approximately 200 mL of blood collected over the 31-week study period. An additional 70 mL blood will be collected from subjects who complete the iron absorption substudy.

13.3. Pharmacokinetic Blood Sample Collection and Processing

See the Laboratory Manual for blood sample labeling, processing, storage, and shipment instructions.

14. ASSESSMENT OF SAFETY

14.1. Safety Parameters

Safety will be assessed by the following parameters:

- ECGs, clinical laboratory tests including CBC (Appendix 1), and physical examinations findings (including vital signs).
- Adverse events and concomitant medications will be evaluated throughout the course of the study.
- Adverse events of interest will be assessed and reported separately and include the following: hematological toxicity > Grade 3 (CTCAE criteria, Version 5.0).

Vital signs will include blood pressure (systolic and diastolic), heart rate, respiratory rate, and temperature.

Electrocardiograms will include ventricular rate, PR, QRS, QT, RR and QTcF.

The specific timing of safety assessments is presented in Table 2.

14.2. Adverse Events and Serious Adverse Events

In this study, AEs and SAEs will be reported in all subjects from the time the subject signs the ICF until the completion of the safety follow-up/end of study visit. Treatment-emergent AEs and SAEs will be evaluated from study drug dosing until the end of study visit.

14.2.1. Definition of Adverse Event

An AE is any event, side effect, or other untoward medical occurrence that occurs in conjunction with the use of a medicinal product in humans, whether or not considered to have a causal relationship to this treatment. An AE can, therefore, be any unfavorable and unintended sign (that could include a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. An AE may include an increase in severity of a pre-existing condition included in a subject's medical history, and symptoms or clinical sequelae of a suspected overdose. "Lack of efficacy" per se will not be reported as an AE or SAE. However, the signs and symptoms and/or clinical sequelae resulting from lack of efficacy will be reported if they fulfil the definition of an AE or SAE.

The Investigator should attempt to establish a diagnosis of the event based on the signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE (and SAE if serious) and not the individual signs/symptoms.

14.2.1.1. Severity of an Adverse Event

Severity of adverse events will be assessed and reported following CTCAE Version 5.0 criteria (National Cancer Institute 2010). Severity of adverse events that do not match CTCAE criteria will be assessed as follows:

- **Mild (Grade 1):** awareness of sign or symptom but easily tolerated

- **Moderate (Grade 2):** discomfort enough to cause interference with usual activity
- **Severe (Grade 3):** incapacitating with inability to work or do usual activity
- **Life-threatening (Grade 4):** could reasonably lead to death without medical intervention
- **Fatal (Grade 5)**

14.2.1.2. Relationship of an Adverse Event to Study Drug

The Investigator will assess the relationship between study drug and the occurrence of each AE. The investigator's assessment of the relationship of each AE to study drug will be recorded in the source documents and the CRF. Alternative causes, such as medical history, concomitant therapy, other risk factors, and the temporal relationship of the event to the study drug should be considered and investigated, if appropriate. The following definitions are general guidelines to help assign grade of attribution:

- **Not related:** The event is clearly related to other factors such as the subject's clinical state, therapeutic interventions or concomitant drugs administered to the subject. This is especially so when an event occurs prior to the commencement of treatment with the study drug.
- **Unlikely:** The event was most likely produced by other factors such as the subject's clinical state, therapeutic interventions, or a concomitant drug administered to the subject and does not follow a known response to the study drug.
- **Possibly:** The event follows a reasonable temporal sequence from the time of study drug administration or follows a known response to the study drug but could have been produced by other factors such as the subject's clinical state, other therapeutic interventions, or concomitant drugs administered to the subject.
- **Related**: The temporal relationship is compelling between the administration of the study drug and the AE cannot be explained by the subject's medical condition or other therapies.

14.2.1.3. Action Taken with Study Medications

Action taken with study medications will be recorded on the AE CRF page, as follows, regardless if a particular category is possible or not in this study:

- Dose Increased
- Dose Not Changed
- Dose Reduced
- Dose Interrupted
- Dose Withdrawn
- Not Applicable
- Unknown

14.2.1.4. Outcome of an Adverse Event

Outcome of an AE will be recorded on the AE CRF as follows:

- Recovered/Resolved
- Recovering/Resolving
- Recovered/Resolved with Sequelae
- Not Recovered/Not Resolving
- Fatal
- Unknown

14.2.2. Definition of Serious Adverse Event

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.

Important medical events that are not one of the above may be considered to be SAEs by the investigator when, based upon appropriate medical judgement, they are considered to be clinically significant and may jeopardize the subject, or when medical or surgical intervention may be required to prevent one of the outcomes listed above.

An AE is considered "life-threatening" if, in the opinion of either the Investigator or the Sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death.

It is important to distinguish between serious and severe AEs. Severity is a measure of intensity, whereas seriousness is defined by the criteria under Section 14.2.2. An AE of severe intensity may not be considered serious.

14.2.3. Pregnancy

The Investigator must report to the Sponsor any pregnancy occurring in a study subject (or subject's partner) during the subject's participation. The report should be submitted within the same timelines as an SAE, although a pregnancy per se is not considered an SAE unless the birth results in a congenital anomaly/birth defect or there is suspicion that the study medication may have interfered with the effectiveness of a contraceptive medication or method. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs.

A pregnancy should be followed-up until reporting of the pregnancy's outcome and the birth date.

As information is available, a pregnancy diagnosed during the study will be reported immediately to the Investigator and Sponsor (or designee), including pregnancy in female partners of male subjects. The pregnancy will be followed to term or outcome and this outcome will be reported to the Sponsor.

14.2.4. Other Safety Parameters and Related Information

Additional safety parameters (laboratory tests including CBC, 12-lead ECGs, physical examinations, and vital signs) and concomitant medications are collected as shown in the Schedules of Assessments in Table 2. Clinical laboratory assessments are listed in Appendix 1.

Abnormal findings, other than pharmacodynamic markers, that are deemed clinically significant or are associated with signs and/or symptoms must be recorded as AEs (or recorded as an SAE if they meet the criteria of being serious) as described previously.

The Investigator should exercise his/her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant. Usually, the abnormality should be associated with a clinically evident sign or symptom.

A clinically significant laboratory abnormality in the absence of clinical signs and symptoms may jeopardize the subject and may require intervention to prevent immediate consequences. For example, a markedly low serum glucose concentration may not be accompanied by coma or convulsions, yet be of a magnitude to require glucose administration to prevent such sequelae.

14.3. Recording Adverse Events

Adverse events spontaneously reported by the subject and/or in response to an open question from the study personnel or revealed by observation will be documented in the medical record in accordance with the Investigator's normal clinical practice and on the AE page of the case report form (CRF). SAEs that occur during the study must be documented in the subject's medical record, in the AE CRF, and on the SAE form.

If there is evidence of an AE or SAE through report or observation, the Investigator or designee will evaluate further and record the following information:

- Time of onset and resolution
- Severity
- Causality/relation to study treatment
- Action taken regarding study drug
- Outcome

14.4. Reporting Adverse Events

Adverse Events: All AEs, regardless of their relationship to PTG-300, will be reported on the AE Case Report Form. In order to ensure timely safety oversight of the trial, AEs should be reported routinely. All reports should include time of onset and resolution, severity (see Section 14.2.1.1), relationship to PTG-300, action taken regarding PTG-300 and outcome.

SAEs: In order to meet the requirements for expedited reporting of SAEs to applicable regulatory authorities and IRBs, all SAEs, must be reported to the Sponsor within 24 hours from the time the site investigational team first becomes aware of the event. This report may be initially achieved by telephone, or by completing a SAE report form and sending it to the Sponsor via the contact information printed on the form.

The SAE report form should be completed as thoroughly as possible and signed by the Investigator before transmittal to the study Sponsor. It is very important that the Investigator provide an assessment of the causal relationship between the event and study drug at the time of the initial report, as this will be useful for submissions to regulatory authorities.

Initial notification of an SAE by telephone must be confirmed in writing 24 hours from the time the site investigational team first become aware of the event using the SAE report form as described above.

As further information regarding the SAE becomes available, such follow-up information should be documented on a new serious adverse event report form and marked as a follow-up report.

Protagonist is responsible for notifying the relevant regulatory authorities of certain events. It is the Principal Investigator's responsibility to notify the IRB or IEC of all SAEs that occur at his or her site. Investigators will also be notified of all unexpected, serious, drug-related events (7/15 Day Safety Reports) that occur during the clinical trial. Each site is responsible for notifying its IRB or IEC of these additional SAEs.

14.5. Follow-Up of Adverse Events and Serious Adverse Events

All AEs and SAEs that are deemed possibly related or probably related to PTG-300 must be followed until resolution, until the condition stabilizes, until the event is otherwise explained, or until the subject dies or is lost to follow-up. The Investigator is responsible to ensure that follow-up includes any supplemental investigations as may be indicated to elucidate as completely as practical the nature and/or causality of the AE/SAE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

If a subject dies during participation in the study or during a recognized follow-up period, the Sponsor should be provided with a copy of any post-mortem findings, including histopathology.

15. STATISTICS

Summary statistics for all efficacy, safety, and PK/PD data, along with the estimation of PTG-300's treatment effect, will be provided. A detailed statistical analysis plan (SAP) will be prepared prior to performing any analysis. In case of differences between the protocol and the SAP, the SAP will take precedence.

15.1. Study Design and Sample Size Estimation

This is a multicenter, single-arm, open-label study.

This trial is exploratory and no rigid criteria for sample size are pre-specified. The choices are based on risk/benefit assessment of accumulating results. However, there will be at least 80% power for a sample size of 20 subjects to detect a 50% reduction in the number of phlebotomies from baseline. The power calculation is based on a hypothesized effect size of 0.5 (mean change = 1, with SD = 2) over 24 weeks using a 1-sided one-sample t-test at alpha=10% level. Twenty-eight (28) subjects will be enrolled in the study, assuming an approximate 25% dropout.

15.2. Demographics/Baseline Comparability

The demographics and baseline characteristics will be summarized by groups, with and without the iron absorption substudy, respectively, and overall using descriptive statistics. Demographics information includes age, sex, race, and ethnicity. Baseline disease characteristics will be included if necessary.

15.3. Data Presentation

Serum chemistries and measurements related to iron studies will be obtained at the clinical site and/or at a central laboratory. The central laboratory value will be used for data presentation and study endpoints. The clinical laboratory value may be used for clinical decision making.

15.4. Definition of Baseline

For the phlebotomy frequency, the subjects' pre-study phlebotomy history will serve as the baseline. For serum ferritin and TSAT, measurements obtained during the screening period will serve as the baseline.

15.5. Analysis of Efficacy Data

Efficacy data will be analyzed for all the subjects included in the Intent-to-Treat Analysis Set defined below.

In general, continuous endpoints will be summarized by standard descriptive statistics (mean, standard deviation, median, minimum, and maximum). Categorical endpoints will be summarized by frequencies and percentages.

Hypothesis testing will be performed by the t-test for each of the change from baseline endpoints listed below to evaluate if the change is significantly different from zero. P-value will not be adjusted for multiple comparisons since this is an early phase safety and proof of concept study. In addition, the point estimate (i.e., mean and/or median) and the confidence interval associated

with the estimate will be provided. Kaplan-Meier estimate will be applied for the time to first phlebotomy data.

Data collected for subjects electing to participate in the iron absorption substudy will be analyzed separately in addition to the analyses for all the Intent-to-Treat subjects. Serum iron, ^{57}Fe, TSAT, serum ferritin, sTfR, and transferrin pertaining to the iron absorption substudy will be analyzed for the subgroup as defined as Iron Absorption analysis set below.

15.6. Analysis Populations

15.6.1. Safety Analysis Set

The Safety Analysis set will include all subjects who receive any amount of study drug.

15.6.2. Intent-to-Treat Analysis Set

The Intent-to-Treat (ITT) Analysis Set for efficacy analyses includes all subjects who received any study drug and have a baseline and at least one post-baseline efficacy assessment.

15.6.3. Iron Absorption Analysis Set

The Iron Absorption (IA) Analysis Set will include all ITT subjects who complete Iron Absorption B.

15.7. Handling of Missing Data

Missing data will not be imputed for calculation of mean changes and other quantitative analyses since the aim for those analyses is to estimate mean changes and other quantitative outcomes in subjects who remain on treatment. Missing data are assumed missing at random for subjects remaining on treatment at each respective time point.

15.8. Analysis of Safety

The safety analysis will include all subjects who receive any amount of study medication. Reported AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). All AEs will be summarized by system organ class and preferred terms within system organ class for overall AEs, for AEs any time during the study. The severity, seriousness, and relationship to study medication will also be summarized in a similar manner. Each AE (based on preferred term) is counted once for a given subject. If the same AE occurred on multiple occasions, the highest severity and least complimentary relationship will be assumed.

The incidence of TEAEs and SAEs will be summarized overall and by the subgroup alone, respectively.

Additionally, laboratory test data, physical examinations, vital signs, and ECGs will be summarized overall and by the subgroup alone, respectively.

15.9. Analyses of Pharmacokinetics and Pharmacodynamics

PK: Concentrations of PTG-300 by dose regimen will be estimated. In addition, concentrations will be reported by time point.

PD: ^{57}Fe absorption will be estimated using the AUC in the subgroup of subjects who complete both iron absorption treatments. Change from baseline in serum iron parameters (serum iron, transferrin, serum ferritin, TSAT) and sTfR will be estimated.

In general, descriptive statistics, including the numbers subjects [n], means, standard deviations [SD], medians, minimums, maximums will be provided for PK parameters as well as for the PD parameters.

15.10. Exploratory Endpoints

- Effects of ADA on safety and efficacy.
- The relationship between PTG-300 pharmacokinetics and pharmacodynamics and efficacy will be explored.

16. ADMINISTRATIVE PROCEDURES AND ETHICAL CONSIDERATIONS

16.1. Study Monitoring

At intervals during the study, as well as after the completion of subject enrollment, the study center will be monitored by a clinical research associate (CRA) for compliance, which will include ensuring that study procedures are being followed, ensuring that accurate and complete data are recorded on CRFs, and reviewing source documentation and drug accountability records. The study will be conducted according to the principles of GCP.

16.2. Audits and Inspections

Authorized representatives of Protagonist, a regulatory authority, an Independent Ethics Committee or an Institutional Review Board may visit the site to perform audits or inspections, including source data verification. The purpose of a Protagonist audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice guidelines of the International Conference on Harmonization (ICH E6 (R2)), and any other applicable regulatory requirements in the country where the trial takes place. The investigator should contact Protagonist immediately if contacted by a regulatory agency about an inspection.

16.3. Institutional Review Board (IRB) Approval

Prior to commencement of the study, written approval is required by the relevant IRB responsible for the investigational site.

16.4. Guidelines for Good Clinical Practice

This study will be conducted in accordance with principles of Good Clinical Practice (GCP) as promulgated by the ICH, and applicable regulatory requirements in the country where the trial takes place. Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording, and reporting trials that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of human subjects are protected under current ethical principles, and that the clinical trial data are credible. Current GCP standards may be found in ICH Guidance E6 (R2). This guidance describes the principles of GCP and the obligations of the institutional review board (IRB), the Investigator and the Sponsor in conducting this study in accordance with those principles.

16.5. Ethics

16.5.1. Ethics Review

The final study protocol, including the final version of the Informed Consent Form, must be approved or given a favorable opinion in writing by an IRB or IEC as appropriate. Prior to the

commencement of the study, written approval will be required by the relevant IRB/IEC responsible for the investigational site.

In addition, the IRB or IEC must approve all advertising used to recruit subjects for the study. The investigator must submit written approval to Protagonist before he or she can enroll any patient/subject into the study.

The Principal Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. The protocol must be re-approved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

The Principal Investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational product. Protagonist will provide this information to the Principal Investigator.

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB or IEC according to local regulations and guidelines.

16.5.2. Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice and applicable regulatory requirements.

16.5.3. Written Informed Consent

A properly executed, written Informed Consent Form, in compliance with the Declaration of Helsinki, ICH GCP, and United States (US) Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 46, Subpart A), will be explained to and signed by each subject prior to entering the trial. The Investigator will provide a copy of the signed Informed Consent Form to each subject and will maintain a copy in the subject's record file.

16.6. Termination of Study

The Sponsor has the right to terminate this study and remove all study material from the site at any time for medical or administrative reasons. In this event, the Sponsor will endeavor to give adequate notice to allow safe withdrawal of subjects from the study.

16.7. Case Report Forms

Site personnel should collect and record data for the study as source documents and transfer the data into the CRF.

The Investigator must ensure that complete data for the clinical study are collected and accurately documented in the appropriate sections of the CRF and adequately supported by the appropriate source documentation. In addition, it is the Investigator's responsibility to provide signatures where requested indicating concurrence with data in the CRF.

16.8. Inspection of Records

Protagonist will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The Investigator agrees to allow the monitor to inspect the drug storage area, study drug stocks, drug accountability records, subject charts and study source documents, and other records relative to study conduct.

16.9. Retention of Records

International Conference on Harmonization, GCP, and US Food and Drug Administration (FDA) guidelines require that essential documents be retained until at least 2 years after the last approval of a marketing application and until there are no pending or contemplated marketing applications, or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product.

However, the essential documents should be retained for a longer period if required by the applicable regulatory requirements or by an agreement with the Sponsor. Records should never be destroyed without written approval from the Sponsor.

If an Investigator leaves the institution, he/she must transfer responsibilities for record retention to another individual willing to accept them. The Investigator must notify the Sponsor in writing of the transfer of study documents before the transfer of the study.

17. PUBLICATION POLICY

The data generated in this clinical study are the exclusive property of Protagonist Therapeutics Inc. and are confidential. Any publication of the results of this study must be authorized by the Protagonist and Protagonist will have the opportunity to review any publications that arise from the investigators before submission for publication.

Publication and reporting of results and outcomes of this trial will be accurate and honest, undertaken with integrity and transparency and in accordance with Protagonist's Publication Policy. Subject confidentiality will be maintained by referring to individual subjects by their identifying code used in the trial. Publication of results will be subjected to fair peer-review.

Authorship on any publication of the results from this study will be based on contributions to study design, enrolment, data analysis, and interpretation of results according to International Committee of Medical Journal Editors guidelines (http://www.icmje.org). All conflicts arising through disputes about authorship will be reviewed by Protagonist.

Acknowledgment will be given to collaborating institutions and hospitals and other individuals and organizations providing finance or facilities.

In the case of no publication, information will only be released to the public and media in accordance with Protagonist's Policy.

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19. APPENDICES

APPENDIX 1. CLINICAL LABORATORY TESTS

HEMATOLOGY/CBC

hemoglobin	% lymphocytes	absolute lymphocytes
hematocrit	% monocytes	absolute monocytes
red blood cell count	% basophils	absolute basophils
white blood cell count	% eosinophils	absolute eosinophils
% neutrophils	absolute neutrophils	platelet count

CHEMISTRY

sodium	calcium	indirect bilirubin
potassium	phosphorous	alkaline phosphatase
chloride	albumin	alanine aminotransferase (ALT, SGPT)
carbon dioxide	total protein	aspartate aminotransferase (AST, SGOT)
blood urea nitrogen (BUN)	uric acid	lactate dehydrogenase (LDH)
creatinine	total bilirubin	
glucose	direct bilirubin	

URINALYSIS

pH	ketones	protein
specific gravity	microscopic exam (RBC and WBC, only when indicated)	
blood	leukocyte esterase	
glucose		

PREGNANCY TEST

Serum pregnancy test (to be completed on site) for female subjects of childbearing potential at Screening and at Safety Follow-Up (Week 29)

Urine pregnancy test (to be completed on site) for female subjects of childbearing potential.

APPENDIX 2. ADDITIONAL LABORATORY TESTS

The following samples to be collected as per Schedule of Assessments (Table 2).

IRON PARAMETERS (PHARMACODYNAMIC PARAMETERS)

serum iron

serum ferritin

transferrin

TSAT

ADDITIONAL ASSESSMENTS

sTfR

hepcidin

ADA

APPENDIX 3. MEDICAL OUTCOME STUDY QUESTIONNAIRE SHORT FORM HEALTH SURVEY (SF-36)

Your Health and Well-Being

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.
Thank you for completing this survey!

For each of the following questions, please mark an ☒ in the one box that best describes your answer.

1. In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

2. <u>Compared to one year ago</u>, how would you rate your health in general <u>now</u>?

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

3. The following questions are about activities you might do during a typical day. Does <u>your health now limit you</u> in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
a Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	☐ 1	☐ 2	☐ 3
b Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	☐ 1	☐ 2	☐ 3
c Lifting or carrying groceries	☐ 1	☐ 2	☐ 3
d Climbing several flights of stairs	☐ 1	☐ 2	☐ 3
e Climbing one flight of stairs	☐ 1	☐ 2	☐ 3
f Bending, kneeling, or stooping	☐ 1	☐ 2	☐ 3
g Walking more than a mile	☐ 1	☐ 2	☐ 3
h Walking several hundred yards	☐ 1	☐ 2	☐ 3
i Walking one hundred yards	☐ 1	☐ 2	☐ 3
j Bathing or dressing yourself	☐ 1	☐ 2	☐ 3

4. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Cut down on the amount of time you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b Accomplished less than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c Were limited in the kind of work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d Had difficulty performing the work or other activities (for example, it took extra effort)	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

5. During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Cut down on the amount of time you spent on work or other activities	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b Accomplished less than you would like	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c Did work or other activities less carefully than usual	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

6. During the <u>past 4 weeks</u>, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

Not at all	Slightly	Moderately	Quite a bit	Extremely
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

7. How much <u>bodily</u> pain have you had during the <u>past 4 weeks</u>?

None	Very mild	Mild	Moderate	Severe	Very severe
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

8. During the <u>past 4 weeks</u>, how much did <u>pain</u> interfere with your normal work (including both work outside the home and housework)?

Not at all	A little bit	Moderately	Quite a bit	Extremely
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks…

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a. Did you feel full of life?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. Have you been very nervous?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. Have you felt so down in the dumps that nothing could cheer you up?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. Have you felt calm and peaceful?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
e. Did you have a lot of energy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
f. Have you felt downhearted and depressed?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
g. Did you feel worn out?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
h. Have you been happy?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
i. Did you feel tired?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?

All of the time	Most of the time	Some of the time	A little of the time	None of the time
☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

11. How TRUE or FALSE is <u>each</u> of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
a. I seem to get sick a little easier than other people	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b. I am as healthy as anybody I know	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c. I expect my health to get worse	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
d. My health is excellent	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

Thank you for completing these questions!

APPENDIX 4. PATIENT GLOBAL IMPRESSION OF CHANGE (PGI-C)

The subject will independently rate the following question of Patient Global Impression of Change (PGI-C) based on his/her overall impression at Week 9 and Week 25.

Patient Global Impression of Change:

Compared to your condition prior to your starting on this study, how much has your condition changed with your current treatment?

❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	❑ 6	❑ 7
Very Much Worse	Much Worse	Minimally Worse	No Change	Minimally Improved	Much Improved	Very Much Improved