A Phase 2a, Randomized, Open-Label Study to Evaluate the Efficacy, Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of ISIS 702843 Administered Subcutaneously to Patients With Non-Transfusion Dependent β-Thalassemia Intermedia

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22 May 2023

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IONIS PHARMACEUTICALS, INC.

ISIS 702843-CS2

A Phase 2a, Randomized, Open-Label Study to Evaluate the Efficacy, Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of ISIS 702843 Administered Subcutaneously to Patients with Non-Transfusion Dependent β-Thalassemia Intermedia

Protocol Amendment 2 – 17 May 2021

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ISIS 702843-CS2

Protocol Amendment 2

Clinical Phase: 2a

A Phase 2a, Randomized, Open-Label Study to Evaluate the Efficacy, Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of ISIS 702843 Administered Subcutaneously to Patients with Non-Transfusion Dependent β-Thalassemia Intermedia

Protocol History:

Original Protocol: 9 August 2019

Amendment 1: 8 September 2020

Sponsor: Ionis Pharmaceuticals, Inc.
Carlsbad, CA 92010

[redacted]

Confidentiality Statement

Protocol Signature Page

Protocol Number:	ISIS 702843-CS2
Protocol Title:	A Phase 2a, Randomized, Open-Label Study to Evaluate the Efficacy, Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of ISIS 702843 Administered Subcutaneously to Patients with Non-Transfusion Dependent β-Thalassemia Intermedia
Amendment:	Protocol Amendment 2
Date:	17 May 2021

I hereby acknowledge that I have read and understand the attached clinical protocol, entitled "A Phase 2a, Randomized, Open-Label Study to Evaluate the Efficacy, Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of ISIS 702843 Administered Subcutaneously to Patients with Non-Transfusion Dependent β-Thalassemia Intermedia," dated 17 May 2021, and agree to conduct the study as described herein.

I agree to comply with the International Conference on Harmonization Tripartite Guideline on Good Clinical Practice.

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Ionis Pharmaceuticals, Inc.

Investigator's Signature

Investigator's Name *(please print)* Date (DD Month YYYY)

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Protocol Number: ISIS 702843-CS2

Protocol Title: A Phase 2a, Randomized, Open-Label Study to Evaluate the Efficacy, Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of ISIS 702843 Administered Subcutaneously to Patients with Non-Transfusion Dependent β-Thalassemia Intermedia

Amendment Number: 2

Amendment Date: 17 May 2021

The following modifications to Protocol ISIS 702843-CS2, Amendment 1 have been made.

Minor changes (not included in the list of changes below) have been made throughout the protocol to correct errors and/or to improve the overall clarity of the original protocol, but these changes do not impact subject safety, exposure, or the overall study design.

The following table provides a summary list of major changes to the protocol:

Protocol Section	Description of Change	Rationale
Section 3.1 Study Design)	Planned ISIS 702843 dose levels are increased as follows: • Cohort A: from 30 mg to 120 mg • Cohort B: from 50 mg to 120 mg • Cohort C: from 80 mg to 120 mg for 3 consecutive doses, followed by 160 mg per dose, if dose escalation is allowed based on a demonstration of adequate safety. Dose escalation will be carried out on an individual patient basis, and only after clinical and laboratory safety evaluations related to the prior dose (scheduled to be 28 days prior) have been completed, and there is an acceptable safety profile as determined by the Principal Investigator (PI) and the Sponsor Medical Monitor, as described in this section.	Safety profiles have been acceptable to date, and increased dose levels may provide additional therapeutic benefit.

Protocol Section	Description of Change	Rationale
[redacted]		
Section 8.1 (ISIS 702843 Administration)	Changed the planned dose levels, the corresponding volumes to be administered, and total dose as shown in	The higher dose levels mentioned above increase the volumes to be administered and
[redacted]		
Section 3.1 (Study Design), Section 3.3 (Number of Patients), and Section 10.2 (Sample Size Considerations)	The number of evaluable patients is decreased from approximately 36 overall to approximately 24 overall, with the number per cohort changed from approximately 12 each to approximately 12 for Cohorts A and B combined, and approximately 12 for Cohort C.	Twelve (12) patients is considered sufficient to evaluate each planned dose level, and there are now only 2: 120 mg per 28-day interval (Cohorts A and B) and 160 mg per 28-day interval (Cohort C, if dose escalation is allowed based on a demonstration of adequate safety).
Section 8.7 (Adjustment of ISIS 702843 Dose and/or Treatment Schedule) and Section 10.5 (Interim Analyses)	Changed the thresholds for the number of patients needed for the interim analyses from at least 30 to at least 20 for the first interim analysis, and from at least 24 to at least 16 for the second interim analysis.	Decreased both thresholds by the same proportion as for the overall number of evaluable patients (see prior row).
Section 5 (Patient Eligibility)	Added that the mean of all values obtained during the Screening Period is generally considered sufficient when making a decision regarding eligibility; however, the Medical Monitor will also consider trends in the data and the significance of one or more individual values that are outside of acceptable limits when making a determination.	Clarity.

Protocol Section	Description of Change	Rationale
Section 5.1 (Inclusion Criteria)	Inclusion Criterion 5 is changed as follows: Mean hemoglobin (Hb) acceptable range is widened from 6.0–10.0 g/dL to 6.0–10.5 g/dL.	An upper limit of 10.0 g/dL is now considered too restrictive.
Section 5.2 (Exclusion Criteria)	Exclusion Criterion 6 (EC#6) is changed as follows: Platelet count Screening laboratory results acceptable range is widened from lower limit of normal (LLN) – 1,000,000/mm^3 to 120,000/mm^3 – 1,000,000/mm^3.	[redacted]
Section 5.2 (Exclusion Criteria)	Exclusion Criterion 20 reworded to provide clarity regarding human immunodeficiency virus (HIV), hepatitis C, or hepatitis B infection that is exclusionary.	Clarity.
Section 5.2 (Exclusion Criteria)	Exclusion Criterion 29 added to provide clarity regarding SARS-CoV-2 infection that is exclusionary, and to clarify that it is acceptable to receive COVID-19 vaccinations while in this study, including in the Screening Period.	Clarity.

Protocol Section	Description of Change	Rationale
Section 8.6.1 (Temporary Stopping Rules for Hb Results)	(i) Criterion 2 changed from confirmed Hb value ≥ 12.0 g/dL to ≥ 12.5 g/dL, and (ii) Criterion 3 changed from confirmed Hb increase of ≥ 2.0 g/dL within a 4-week period and Hb > 10.0 g/dL, to the same except Hb > 10.5 g/dL.	Both of these increases of 0.5 g/dL correspond with the change to Inclusion Criterion 5 (IC#5), which is an increase of 0.5 g/dL to the upper limit of mean Hb (from 10.0 to 10.5 g/dL; see above). This maintains (i) a difference of 2.0 g/dL between the Criterion 2 value and the upper limit of mean Hb in IC#5, and (ii) no difference between the Criterion 3 value and the upper limit of mean Hb in IC#5.
Section 8.6.6 (Temporary and Permanent Stopping Rules for Platelet Count Results)	A statement is added to clarify that in the event of any platelet count > 1,000,000/mm^3, the utility of adjustments to concomitant medications (e.g., anti-coagulants) will be determined by the PI in consultation with the Medical Monitor.	Clarity.
Section 8.7 (Adjustment of ISIS 702843 Dose and/or Treatment Schedule)	Except for the reference to the second interim analysis and within criterion 4, all occurrences of Day 365 (Week 53) have been changed to Day 281 (Week 41) to allow dose adjustments for the stated reasons to occur earlier. Within Criterion 4, added Day 281 (Week 41).	Earlier increases in dose levels may provide additional therapeutic benefit.
Section 8.7 (Adjustment of ISIS 702843 Dose and/or Treatment Schedule)	Added to Criteria 1-4 the additional dose adjustments that are now possible.	Maximum dose per 28-day interval is increased from 120 mg to 160 mg (see above).
Section 3.3 (Number of Patients) and Section 4.3 (Replacement of Patients)	Changed the maximum number of replacements from 3 patients per cohort (i.e., 9 total) to 3 patients for Cohorts A and B combined, and 3 patients for Cohort C (i.e., 6 total).	Three (3) replacements per dose level is considered sufficient, and there are now only two planned: 120 mg per 28-day interval (Cohorts A and B) and 160 mg per 28-day interval (Cohort C, if dose escalation is allowed based on a demonstration of adequate safety).

Protocol Section	Description of Change	Rationale
Section 3.3 (Number of Patients) and Section 4.3 (Replacement of Patients)	Decreased the maximum enrollment for the study from 45 to 30 patients.	The number of evaluable patients is decreased from approximately 36 overall to approximately 24 overall (see above) and the maximum number of replacements is decreased from 9 total to 6 total (see prior row).
Section 3.3 (Number of Patients) and Section 4.3 (Replacement of Patients)	Clarified that the Sponsor may close enrollment to one cohort before another, and that it is likely that enrollment to Cohort B will be closed before enrollment to Cohort C.	The intent is to have approximately 12 evaluable patients in Cohorts A and B combined, and approximately 12 evaluable patients in Cohort C.
Section 3.3 (Number of Patients) and Section 4.3 (Replacement of Patients)	Removed the statement that patients will not be replaced if the PI, in consultation with the Medical Monitor, concludes that replacement is not advisable for reasons of safety.	This statement is more applicable to a Phase 1 study.
Section 3.2 (Number of Study Centers)	Changed the list of territories to correspond with the centers currently planned for this study; deleted Canada and added Mediterranean countries.	Clarity.
Section 6.2.2 (Laboratory and Imaging Assessments)	Clarified that Investigators may, at their discretion, test specific laboratory parameters that may be prone to clotting, clumping, or hemolysis (e.g., hematology samples) at their local laboratory, in addition to the required central laboratory samples. Also, clarified that in the event of an unreportable platelet count result, repeat testing may be conducted at a central or local laboratory.	To clarify the flexibility there is regarding the allowance for testing at a local laboratory, including repeat platelet count testing
Appendix A (Schedule of Procedures – Screening Period, Treatment Period, & Early Termination from Treatment (ETFT))	Added an "X" in the row for Clinic Visit and the column for SV1.	Correction of an error in Amendment 1.
Appendix A (Schedule of Procedures – Screening Period, Treatment Period, & Early Termination from Treatment (ETFT))	For Echocardiogram and DEXA scan, changed the acceptable timing within the Screening Period from SV1 to anytime through SV2.	To provide more flexibility with respect to when these assessments need to be performed to establish Baseline.

PROTOCOL SYNOPSIS

Protocol Title	A Phase 2a, Randomized, Open-Label Study to Evaluate the Efficacy, Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of ISIS 702843 Administered Subcutaneously to Patients with Non-Transfusion Dependent β-Thalassemia Intermedia
Study Phase	2a
Indication	Anemia and Iron overload in patients with Non-Transfusion Dependent β-Thalassemia Intermedia
Primary Objective	Evaluate the efficacy of antisense inhibitor of TMPRSS6 (ISIS 702843) by demonstrating an improvement in hemoglobin (Hb) concentration at Week 27 of treatment
Secondary Objectives	Evaluate the efficacy of ISIS 702843 by demonstrating an improvement in Hb concentration at Week 53 of treatment Evaluate the efficacy of ISIS 702843 by demonstrating an improvement in liver iron concentration (LIC) at Week 53 of treatment
Exploratory Objectives	Evaluate the impact of ISIS 702843 on Ineffective Erythropoiesis (IE): • Erythroferrone (ERFE); soluble transferrin receptor 1 (sTfR1); reticulocyte count; reticulocyte Hb content (CHr); erythropoietin (EPO); growth differentiation factor 15 (GDF15); erythroblast count; bone marrow iron concentration Evaluate the impact of ISIS 702843 on measures of iron overload: • LIC; spleen iron concentration; serum transferrin saturation (TSAT); non-transferrin-bound iron (NTBI); serum ferritin Evaluate the efficacy of ISIS 702843 by demonstrating an increase in serum hepcidin concentration Evaluate the efficacy of ISIS 702843 by demonstrating improvement in anemia parameters: • Hb; red blood cell (RBC) count; hematocrit; MCV; MCH; MCHC Evaluate the impact of ISIS 702843 on hemolysis: • Haptoglobin; protoporphyrin-IX; bilirubin (indirect); lactate dehydrogenase (LDH); peripheral blood smear Evaluate the impact of ISIS 702843 on coagulation profile: • Prothrombin time (PT); international normalized ratio (INR); activated partial thromboplastin time (aPTT); antithrombin III; Protein S; Protein C Evaluate the impact of ISIS 702843 on disease severity and quality of life measures: • Number of transfusions; chelator usage; non-transfusion dependent β-thalassemia patient-reported outcomes (NTDT-PRO©); 36-item short form survey (SF-36); Patient Global Impression of Change (PGIC); Investigator's Static Global Assessment (ISGA); Functional Assessment of Chronic Illness Therapy – Fatigue (FACIT-Fatigue)

PROTOCOL SYNOPSIS CONTINUED

Exploratory Objectives Continued	Evaluate the impact of ISIS 702843 on functional status of the patient: • 6-minute walk test (6MWT) with pulse oximetry
Safety Objectives	Evaluate the safety and tolerability of multiple doses of ISIS 702843 in patients with β-Thalassemia Intermedia Evaluate the impact of multiple doses of ISIS 702843 on the emergence and/or progression of recognized complications of β-Thalassemia Intermedia
PK Objectives	Evaluate pharmacokinetic (PK) exposure over time and potential PK/pharmacodynamic (PD) correlation on relevant biomarkers and efficacy outcome measures
Study Design	ISIS 702843-CS2 is a multi-center, randomized, open-label study of subcutaneously (SC) administered ISIS 702843 in patients with non-transfusion dependent β-Thalassemia Intermedia. The study will comprise 3 cohorts – Cohorts A, B, and C. However, upon Amendment 2 approval, (i) the dose level for Cohorts A and B will become the same at 120 mg ISIS 702843, and (ii) no new patients will be randomized to Cohort A. There will be approximately 12 eligible patients in Cohorts A and B combined, and approximately 12 eligible patients in Cohort C, for a total of approximately 24 eligible patients overall. These patients will be stratified based on Screening Mean Hb for Eligibility, low (≤ 8.0 g/dL) and high (> 8.0 g/dL). Then patients will be randomized in parallel to Cohort B (120 mg ISIS 702843 per dose) or Cohort C (120 mg ISIS 702843 per dose for three consecutive doses, followed by 160 mg ISIS 702843 per dose, if dose escalation is allowed based on a demonstration of adequate safety). Dose escalation to 160 mg ISIS 702843 in Cohort C will be done on an individual patient basis, if such escalation is allowed based on the judgment of the Principal Investigator (PI), in consultation with the Medical Monitor, specifically that there is an acceptable safety profile as described in Section 3.1. In addition, ISIS 702843 dose adjustments (decreases and increases to the proposed maximum clinical dose of 160 mg per 28-day interval) are permitted in all cohorts, as described in Section 8.7. Each patient will be treated for up to 2 years, receiving up to 27 doses of ISIS 702843, with a planned 28-day interval between each dose. This is an open-label study. Nevertheless, to facilitate obtaining objective feedback from the patients on their quality of life measures, it is preferable that patients not be informed of how their dose compares to the dosing levels of other cohorts and individual patients.

PROTOCOL SYNOPSIS CONTINUED

Number of Patients	The study will enroll approximately 24 evaluable patients overall, with approximately 12 evaluable patients in Cohorts A and B combined, and approximately 12 evaluable patients in Cohort C. Patients who terminate early from the study may be replaced, with a maximum of 3 replacements for Cohorts A and B combined, and with a maximum of 3 replacements for Cohort C. Patients who discontinue after Day 365 (Week 53) will not be replaced. Each additional patient will be assigned to the same cohort and stratum (i.e., Hb ≤ 8.0 g/dL, or Hb > 8.0 g/dL) as the patient they are replacing. Before a fit is identified, patients who do not qualify for that stratum will still be enrolled in the study, including into that cohort. The maximum enrollment for the study will be limited to 30 patients or until enrollment is closed by the Sponsor. The Sponsor may close enrollment to one cohort before another; for example, it is likely that enrollment to Cohort B will be closed before enrollment to Cohort C because the intent is to have approximately 12 evaluable patients in Cohorts A and B combined, and approximately 12 evaluable patients in Cohort C.
Study Population	The Medical Monitor may be consulted if any questions arise regarding Inclusion or Exclusion criteria listed below. **Inclusion Criteria** 1. Patient must have given written informed consent and be able to comply with all study requirements 2. Aged 18-65 years old, inclusive, at the time of informed consent 3. Clinical diagnosis of β-Thalassemia Intermedia with genotypic confirmation of β-globin gene mutations including but not limited to Hemoglobin E (HbE)/β-thalassemia 4. Patient must be non-transfusion dependent as defined by: No more than 8 transfusions in the past 12-month period, and no transfusions in the 8-week period prior to Day 1 5. Mean Hb within the range 6.0-10.5 g/dL, inclusive, with this mean based on all Hb measurements taken in the Screening Period that are at least 6 weeks after the most recent transfusion for that patient. This mean must be based on at least 2 Hb measurements. 6. LIC within the range of 3.0-20.0 mg Fe/g dry weight, inclusive 7. Chelators will be permitted provided the dose has not been increased for at least 2 months prior to Day 1, with LIC > 5.0 mg Fe/g dry weight and serum ferritin > 300 ng/mL

PROTOCOL SYNOPSIS CONTINUED

Study Population Continued	**Inclusion Criteria Continued** 8. Females must be non-pregnant and non-lactating, and one of the following: (i) surgically sterile (e.g., tubal occlusion, hysterectomy, bilateral salpingectomy, bilateral oophorectomy), (ii) postmenopausal (defined as 12 months of spontaneous amenorrhea without an alternative medical cause and follicle stimulating hormone [FSH] levels in the postmenopausal range for the laboratory involved), (iii) abstinent*, or (iv) if engaged in sexual relations and of child-bearing potential, the patient must be using a highly effective (Section 6.3.1) contraceptive method from the time of signing the informed consent until at least 13 weeks after the last dose of ISIS 702843. Males must be one of the following: (i) surgically sterile, (ii) abstinent*, or (iii) if engaged in sexual relations with a female of child-bearing potential, the patient must be using a highly effective (Section 6.3.1) contraceptive method from the time of signing the informed consent until at least 13 weeks after the last dose of ISIS 702843. * Abstinence is only acceptable as true abstinence, i.e., when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a trial, and withdrawal are not acceptable methods of contraception. **Exclusion Criteria** 1. Genotypic confirmation of either α-globin gene triplication or sickle hemoglobin (HbS)/b-thalassemia, as determined by genetic assessment of a panel of blood-related disorders 2. Clinically significant abnormalities in medical history or physical examination, which at the discretion of the PI will pose significant additional risk to the patient in participating in the study 3. Clinically significant abnormalities in Screening laboratory values that would render a patient unsuitable for inclusion, at the discretion of the PI 4. Current use of iron-chelation therapy if LIC is 3.0–5.0 mg Fe/g dry weight, inclusive, or if serum ferritin ≤ 300 ng/mL 5. Symptomatic splenomegaly, including abdominal pain or organ obstruction, or evidence of hypersplenism, such as low white blood cell (WBC) count and/or low platelets 6. Platelet count Screening laboratory results < 120,000/mm^3 or > 1,000,000/mm^3, or any other clinically significant abnormalities in platelet count Screening laboratory values that would render a patient unsuitable for inclusion

PROTOCOL SYNOPSIS CONTINUED

Study Population Continued	**Exclusion Criteria Continued** 7. Significant concurrent/recent coagulopathy; history of non-traumatic significant bleeding; history of immune thrombocytopenic purpura (ITP); current use of SC anti-coagulants; history of thrombotic events, including stroke or deep vein thrombosis (DVT) 8. Clinically significant renal dysfunction which at the discretion of the PI will pose significant additional risk to the patient in participating in the study 9. Either of the following confirmed manifestations of renal dysfunction: 9a. Estimated glomerular filtration rate (eGFR) < 45 mL/min/1.73 m^2, using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation 9b. Proteinuria manifesting as urine protein-to-creatinine ratio (UPCR) ≥ 0.50 mg/mg 10. Clinically significant liver function test (LFT) abnormalities at the discretion of the PI 11. Alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) > 3.0 × ULN 12. Historical diagnosis of cirrhosis, or current signs and symptoms of cirrhosis 13. Fasting blood glucose > 2.0 × ULN 14. Significant pulmonary hypertension (PHT) defined as tricuspid regurgitation > 3.0 meters per second (m/s) on echocardiography and/or requiring treatment 15. Uncontrolled hypertension (which for this protocol is considered > 140 mm Hg systolic or > 90 mm Hg diastolic) 16. Heart failure class 3 or higher (New York Heart Association, NYHA) 17. Ejection fraction < 50% by echocardiogram, multigated acquisition (MUGA), or cardiac magnetic resonance imaging (MRI) 18. Patients unable to have MRI performed, for example, because of a pacemaker or implantable cardioverter-defibrillator (MRI is being used to measure LIC) 19. Active infection requiring systemic antiviral or antimicrobial therapy that will not be completed prior to Day 1 20. Active infection with human immunodeficiency virus (HIV), hepatitis C, or hepatitis B diagnosed by initial serological testing and confirmed with RNA testing, or prior treatment for hepatitis C. Patients at Screening who test positive by serology, but negative by RNA may be allowed in consultation with the Sponsor Medical Monitor 21. Unwillingness to comply with study procedures, including follow-up, as specified by this protocol, or unwillingness to cooperate fully with the Investigator 22. Recent introduction of hydroxyurea (within 6 months prior to Day 1)

PROTOCOL SYNOPSIS CONTINUED

Study Population Continued	**Exclusion Criteria Continued** 23. Treatment with or exposure to another investigational drug, biological agent, ASO, small interfering ribonucleic acid (siRNA), or device within one month of Screening, or 5 half-lives of investigational agent, whichever is longer; or: • Treatment with or exposure to sotatercept (ACE-011), luspatercept-aamt (Reblozyl®), or ruxolitinib (Jakafi®) within 4 months of Screening • Treatment with or exposure to hematopoietic stimulating agents (e.g., EPOs) or any hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs) (e.g., roxadustat, vadadustat, daprodustat, molidustat, desidustat) within 8 weeks of Day 1 • Prior bone marrow transplant, stem cell transplant, or gene therapy 24. Regular use of alcohol within 6 months prior to Screening [> 7 drinks/wk for females, > 14 drinks/wk for males (1 drink = 5 ounces [150 mL] of wine or 12 ounces [360 mL] of beer or 1.5 ounces [45 mL] of hard liquor)] 25. Surgery associated with significant blood loss within 4 months of Screening, splenectomy within 12 months of Screening, or splenectomy scheduled during the Treatment Period 26. Use of iron supplements, including iron-containing vitamins, within 4 months of Screening 27. Pregnant or lactating 28. Have any other conditions which, in the opinion of the PI, would make the patient unsuitable for inclusion, or could interfere with the patient participating in or completing the study 29. Active infection with the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes COVID-19. (Note: It is acceptable to receive COVID-19 vaccinations while in this study, including in the Screening Period, but it is preferred that there is an interval of at least 7-10 days between each vaccination and ISIS 702843 dose.)

PROTOCOL SYNOPSIS CONTINUED

Treatment Groups

During the 2-year Treatment Period, each patient will receive up to 27 SC doses of ISIS 702843, with a planned 28-day interval between each dose (i.e., Day 1 [Week 1], Day 29 [Week 5], Day 57 [Week 9], …, Day 729 [Week 105]). The planned total ISIS 702843 dose regimens are shown in the table below. However, ISIS 702843 dose may be adjusted as described in Section 8.7. Planned dose levels are listed in the table below, with the planned dose escalation in Cohort C being on an individual patient basis (Section 3.1).

Planned Dose of ISIS 702843	**# of Doses**	**Total ISIS 702843**
Cohort A: 30 mg / [redacted] ISIS 702843 for at least 1 dose (because all Cohort A patients are randomized before Amendment 2 approval); then: 120 mg / [redacted] ISIS 702843	27	3,150 mg (or less, depending on the number of doses before Amendment 2 approval)
Cohort B: 120 mg / [redacted] ISIS 702843	27	3,240 mg (less for patients randomized before Amendment 2 approval)
Cohort C: 120 mg / [redacted] ISIS 702843 for 3 consecutive doses, then 160 mg / [redacted] ISIS 702843 if dose escalation is allowed based on a demonstration of adequate safety	27	4,200 mg (less for patients randomized before Amendment 2 approval)

Study Drug Dosage and Administration

The Sponsor will provide ISIS 702843 [redacted]

Unless the allowances in the circumstance of a public health emergency apply (Section 3.8), through Day 365 (Week 53) all doses will be administered at the clinic, either by Study Center staff or by the trained patient/caregiver under the observation of Study Center staff. Self-administration at home is allowed after Day 365 (Week 53) – earlier if Section 3.8 applies – and after appropriate training of patient/caregiver.

All doses will be administered [redacted] SC injection(s) in the abdomen, thigh, or outer area of the upper arm (Section 8.1).

PROTOCOL SYNOPSIS CONTINUED

Rationale for Dose and Schedule of Administration	[redacted]
	There are multiple reasons for the study duration of 2 years. This duration (i) allows assessment of long-term efficacy and safety, (ii) provides further guidance on dosing and monitoring for subsequent development, (iii) allows evaluation of durability of response, and (iv) permits detection of potential chronic or delayed safety signals.

PROTOCOL SYNOPSIS CONTINUED

Adjustment of Dose and/or Treatment Schedule	Treatment schedule adjustments and dose pauses will be permitted according to predefined criteria (see Section 8.7). Two (2) interim analyses are planned. The first interim analysis is planned to occur when at least 20 patients have completed evaluations associated with Day 183 (Week 27). The second interim analysis is planned to occur when at least 16 patients have completed evaluations associated with Day 365 (Week 53). Based on these and/or other analysis(es), ISIS 702843 dose adjustments in addition to the dose escalation to 160 mg planned in Cohort C (Section 3.1) will be permitted based on prespecified criteria and if the data support such a change providing additional therapeutic benefit or safety to the patient(s). These adjustments may be applicable to: • An entire cohort, but only after the first interim analysis • An individual patient, but only after that individual has completed PK and PD evaluations associated with Day 365 (Week 53) Earlier adjustment(s) for an individual patient may occur if it is deemed necessary, and the PI should discuss these proposed adjustment(s) with the Medical Monitor prior to implementation. Maximum ISIS 702843 dose will not exceed 160 mg per administration, with a planned 28-day interval between each dose. Standard-of-care interventions, including transfusion, introduction of iron chelation therapy, and adjustment of chelator dose or formulation, will be permitted, at the discretion of the PI. For example, if serum ferritin decreases to the extent that over-chelation is a concern, then it is expected that the PI will consider reducing the chelator dose, as appropriate, before consideration is given to adjusting the ISIS 702843 dose.
Study Visit Schedule and Procedures	Detailed information regarding the study procedures is outlined in Appendix A (Schedule of Procedures). Appendix B presents a list of laboratory tests required for the study. Blood and urine samples will be collected regularly throughout the study for efficacy, safety, PK, and PD analyses. MRI evaluations will be performed to monitor LIC, spleen iron concentration, and, if feasible, bone marrow iron concentration. An Iron Metabolism Panel comprising serum biomarkers of iron loading (TSAT, NTBI, ferritin) will complement the MRI assessments, and also includes a key PD biomarker, hepcidin. An Endocrine Panel of serum biomarkers will be evaluated to monitor for iron-related endocrinopathies (diabetes, hypogonadism, hypothyroidism) A panel of serum biomarkers will be analyzed to monitor IE. Biomarkers of hemolysis will also be assessed.

PROTOCOL SYNOPSIS CONTINUED

Study Visit Schedule and Procedures Continued	The safety of ISIS 702843 will be monitored in an ongoing fashion throughout the study via AE reporting, routine clinical assessments, blood testing, and urine testing. In addition, spleen size and volume, and liver size and volume will be monitored. The length of each patient's participation in the study is approximately 29 months that includes an approximately 2-month Screening Period, a 24-month Treatment Period, and a 3-month Post-Treatment Period (PTP). Patients who discontinue from ISIS 702843 treatment and agree to remain in the study will attend the following clinic visits: Early Termination from Treatment (ETFT), PTP Visit 1, PTP Visit 2, and PTP Last Visit. If the patient is unable or unwilling to participate in all of these visits, the PI should determine the follow-up procedures the patient would agree to complete. Every effort should be made to complete the ETFT procedures and observations at the time of withdrawal.
Primary Endpoint	Proportion of patients who have a ≥ 1.0 g/dL increase from Baseline in Hb, comparing the 3 cohorts at Week 27 of treatment.
Secondary Endpoints	Proportion of patients who have a ≥ 1.5 g/dL increase from Baseline in Hb, comparing the 3 cohorts at Week 53 of treatment. Proportion of patients who have a ≥ 1.0 mg Fe/g dry weight decrease from Baseline in LIC, comparing the 3 cohorts at Week 53 of treatment.
Exploratory Endpoints	Impact of ISIS 702843 on IE: • Mean change from Baseline in plasma erythroferrone concentration, comparing the 3 cohorts over time • Mean change from Baseline in plasma sTfR1 concentration, comparing the 3 cohorts over time • Mean change from Baseline in reticulocyte count, comparing the 3 cohorts over time • Mean change from Baseline in CHr, comparing the 3 cohorts over time • Mean change from Baseline in plasma EPO concentration, comparing the 3 cohorts over time • Mean change from Baseline in plasma GDF15 concentration, comparing the 3 cohorts over time • Mean change from Baseline in erythroblast count, comparing the 3 cohorts over time • Mean change from Baseline in bone marrow iron concentration, comparing the 3 cohorts over time

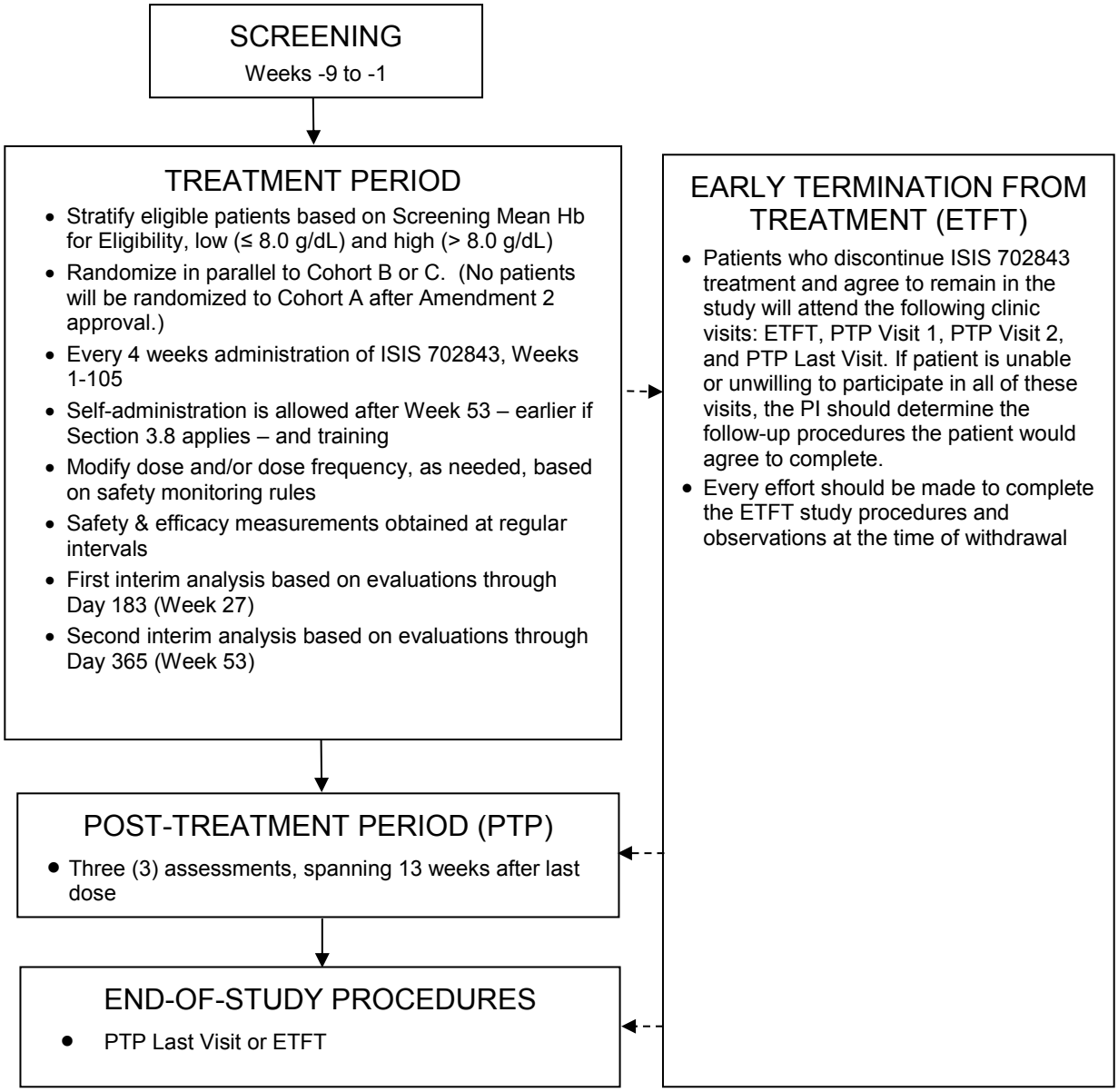
PROTOCOL SYNOPSIS CONTINUED

Exploratory Endpoints Continued	Impact of ISIS 702843 on measures of iron overload: • Mean change from Baseline in LIC, comparing the 3 cohorts over time • Mean change from Baseline in spleen iron concentration, comparing the 3 cohorts over time • Mean change from Baseline in TSAT, comparing the 3 cohorts over time • Mean change from Baseline in NTBI, comparing the 3 cohorts over time • Mean change from Baseline in serum ferritin, comparing the 3 cohorts over time • Mean change from Baseline in chelator usage, comparing the 3 cohorts over time • Mean change from Baseline in hepcidin, comparing the 3 cohorts over time Impact of ISIS 702843 on measures of anemia: • Mean change from Baseline in Hb, comparing the 3 cohorts over time • Mean change from Baseline in RBC count, comparing the 3 cohorts over time • Mean change from Baseline in hematocrit, comparing the 3 cohorts over time • Mean change from Baseline in mean corpuscular volume (MCV), comparing the 3 cohorts over time • Mean change from Baseline in mean corpuscular Hb, amount (MCH), comparing the 3 cohorts over time • Mean change from Baseline in mean corpuscular Hb concentration (MCHC), comparing the 3 cohorts over time Impact of ISIS 702843 on hemolysis: • Mean change from Baseline in plasma haptoglobin, comparing the 3 cohorts over time • Mean change from Baseline in plasma protoporphyrin-IX, comparing the 3 cohorts over time • Mean change from Baseline in plasma bilirubin (indirect), comparing the 3 cohorts over time • Mean change from Baseline in plasma LDH, comparing the 3 cohorts over time • Evaluation of peripheral blood smears, comparing the 3 cohorts over time Impact of ISIS 702843 on coagulation profile: • Mean change from Baseline in PT, comparing the 3 cohorts over time • Mean change from Baseline in INR, comparing the 3 cohorts over time • Mean change from Baseline in aPTT, comparing the 3 cohorts over time • Mean change from Baseline in antithrombin III, comparing the 3 cohorts over time • Mean change from Baseline in Protein S, comparing the 3 cohorts over time • Mean change from Baseline in Protein C, comparing the 3 cohorts over time

PROTOCOL SYNOPSIS CONTINUED

Exploratory Endpoints Continued	Impact of ISIS 702843 on disease severity and quality of life measures: • Change in the number of transfusions, comparing the 3 cohorts over time • Change in chelator usage, comparing the 3 cohorts over time • Mean change from Baseline in NTDT-PRO©, comparing the 3 cohorts over time • Mean change from Baseline in SF-36, comparing the 3 cohorts over time • Mean change from Baseline in PGIC, comparing the 3 cohorts over time • Mean change from Baseline in ISGA, comparing the 3 cohorts over time • Mean change from Baseline in FACIT-Fatigue, comparing the 3 cohorts over time Impact of ISIS 702843 on functional status of the patient: • Mean change from Baseline in 6MWT, comparing the 3 cohorts over time
Safety Endpoints	Adverse events (AEs), vital signs, clinical laboratory tests (serum chemistry, hematology, urinalysis, coagulation panel, thyroid panel), electrocardiogram (ECG). Measures of extramedullary hematopoiesis, liver function, splenomegaly, osteoporosis, diabetes, pulmonary hypertension, and leg ulcers.
PK Endpoints	A PK profile (including pre-dose sampling) over a 6-hour period will be collected at Day 1 (Week 1). Two (2) samples – pre-dose and 3-hour post-dose – will be evaluated at Day 197 (Week 29). Trough levels will be evaluated just prior to each dose through Day 169 (Week 25), as well as on a more limited basis throughout the rest of the Treatment Period. One (1) sample will be evaluated on Day 183 (Week 27); there is no dose at this visit. In the Post-Treatment Period, 1 sample at each of the 3 planned visits will be evaluated.
Statistical Considerations	There is no statistical rationale for the selected sample size of the study dose treatment cohorts. The sample size was based on prior experience to ensure that the safety, tolerability, PK, and PD of ISIS 702843 will be adequately assessed while minimizing unnecessary patient exposure. Eligible patients will be stratified based on Screening Mean Hb for Eligibility, low (≤ 8.0 g/dL) and high (> 8.0 g/dL), and randomized in parallel to Cohort B or C. No patients will be randomized to Cohort A after Amendment 2 approval. Two (2) interim analyses are planned: • The first interim analysis is planned to occur when at least 20 patients have completed evaluations associated with Day 183 (Week 27) • The second interim analysis is planned to occur when at least 16 patients have completed evaluations associated with Day 365 (Week 53)
Sponsor	Ionis Pharmaceuticals, Inc.

STUDY DESIGN AND TREATMENT SCHEMA



STUDY GLOSSARY

Abbreviation	Definition
2′-MOE	2′-*O*-(2-methoxyethyl)
6MWT	6-minute walk test
ADA	anti-drug antibodies
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase (formerly referred to as serum glutamic pyruvic transaminase [SGPT])
ANA	antinuclear antibody
aPTT	activated partial thromboplastin time
ASO	antisense oligonucleotide
AST	aspartate aminotransferase (formerly referred to as serum glutamic oxaloacetic transaminase [SGOT])
βhCG	beta-subunit of human chorionic gonadotropin (pregnancy test)
BP	blood pressure
BUN	blood urea nitrogen
CBC	complete blood count
CHr	reticulocyte hemoglobin content
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CMV	Cytomegalovirus
CRF	case report form
CRNMB	clinically relevant non-major bleeding
CTCAE	Common Terminology Criteria for Adverse Events
DEXA	dual energy X-ray absorptiometry
DVT	deep vein thrombosis
EBV	Epstein-Barr virus
EC	Ethics Committee
ECG	electrocardiogram
eCRF	electronic Case Report Form
EDC	electronic data capture
eGFR	estimated glomerular filtration rate
EPO	erythropoietin
ETFT	early termination from treatment
FACIT	Functional Assessment of Chronic Illness Therapy
FAS	Full Analysis Set
FBS	fasting blood sugar
Fe/g	iron (Fe) per gram of dry weight of liver
FSH	follicle-stimulating hormone
GalNAc	*N*-acetyl galactosamine
GCP	Good Clinical Practice
GDF15	growth differentiation factor 15
GGT	gamma-glutamyltransferase
HAV	hepatitis A virus
Hb	hemoglobin

Abbreviation	Definition
HbE	hemoglobin E
HbS	sickle hemoglobin
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HIF-PHI	hypoxia-inducible factor prolyl hydroxylase inhibitor
HIV	human immunodeficiency virus
HR	heart rate
ICH	International Conference on Harmonization
IE	ineffective erythropoiesis
IgG	immunoglobulin G
IgM	immunoglobulin M
INR	international normalized ratio
IRB	Institutional Review Board
IRT	Interactive Response Technology
ISGA	Investigator's Static Global Assessment
ISIS 702843	antisense inhibitor of TMPRSS6
ITP	immune thrombocytopenic purpura
LDH	lactate dehydrogenase
LFT	liver function test
LH	luteinizing hormone
LIC	liver iron concentration
LLN	lower limit of normal
m^2	square meter
MB	major bleeding
MCH	mean corpuscular hemoglobin (amount)
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MedDRA™	Medical Dictionary for Regulatory Activities
mm^3	cubic millimeter
mm Hg	millimeters of mercury
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
m/s	meters per second
MUGA	multigated acquisition
NCS	not clinically significant
NOAEL	no-observed-adverse-effect-level
NTBI	non-transferrin-bound iron
NTDT	non-transfusion dependent thalassemia
on study	The patient is 'on study' from signing of the informed consent until their last study visit
PD	pharmacodynamic(s)
PGIC	Patient Global Impression of Change
PHT	pulmonary hypertension
PI	Principal Investigator

Abbreviation	Definition
PK	pharmacokinetic(s)
PRO	patient-reported outcome(s)
PT	prothrombin time
PTP	post-treatment period
RBC	red blood cell(s)
RDW	red cell distribution width
RNase H1	an ubiquitous endonuclease that specifically hydrolyzes the RNA strand in RNA/DNA hybrids
SAE	serious adverse event
SAP	Statistical Analysis Plan
SC	subcutaneous(ly)
siRNA	small interfering ribonucleic acid
sTfR1	soluble transferrin receptor 1
Study Day 1	defined as the first day ISIS 702843 is administered to the patient
Study Drug	ISIS 702843 (there is no placebo in this study)
SUSAR	suspected unexpected serious adverse reaction
SV	Screening Visit
TEAE	treatment-emergent adverse event
TMPRSS6	transmembrane protease, serine 6
TSAT	transferrin saturation
TSH	thyroid-stimulating hormone
ULN	upper limit of normal
UPCR	urine protein-to-creatinine ratio
WBC	white blood cell

1. OBJECTIVES AND ENDPOINTS

1.1. Objectives

1.1.1. Primary Objective

Evaluate the efficacy of antisense inhibitor of TMPRSS6 (ISIS 702843) by demonstrating an improvement in hemoglobin (Hb) concentration at Week 27 of treatment.

1.1.2. Secondary Objectives

Evaluate the efficacy of ISIS 702843 by demonstrating an improvement in Hb concentration at Week 53 of treatment.

Evaluate the efficacy of ISIS 702843 by demonstrating an improvement in liver iron concentration (LIC) at Week 53 of treatment.

1.1.3. Exploratory Objectives

Evaluate the impact of ISIS 702843 on Ineffective Erythropoiesis (IE):

- Erythroferrone (ERFE); soluble transferrin receptor 1 (sTfR1); reticulocyte count; reticulocyte Hb content (CHr); erythropoietin (EPO); growth differentiation factor 15 (GDF15); erythroblast count; bone marrow iron concentration

Evaluate the impact of ISIS 702843 on measures of iron overload:

- LIC; spleen iron concentration; serum transferrin saturation (TSAT); non-transferrin-bound iron (NTBI); serum ferritin

Evaluate the efficacy of ISIS 702843 by demonstrating an increase in serum hepcidin concentration.

Evaluate the efficacy of ISIS 702843 by demonstrating improvement in anemia parameters:

- Hb; RBC count; hematocrit; MCV; MCH; MCHC

Evaluate the impact of ISIS 702843 on hemolysis:

- Haptoglobin; protoporphyrin-IX; bilirubin (indirect); lactate dehydrogenase (LDH); peripheral blood smear

Evaluate the impact of ISIS 702843 on coagulation profile:

- Prothrombin time (PT); international normalized ratio (INR); activated partial thromboplastin time (aPTT); antithrombin III; Protein S; Protein C

Evaluate the impact of ISIS 702843 on disease severity and quality of life measures:

- Number of transfusions; chelator usage; non-transfusion dependent β-thalassemia patient-reported outcomes (NTDT-PRO©); 36-item short form survey (SF-36); Patient Global Impression of Change (PGIC); Investigator's Static Global Assessment (ISGA); Functional Assessment of Chronic Illness Therapy – Fatigue (FACIT-Fatigue)

Evaluate the impact of ISIS 702843 on functional status of the patient:

- 6-minute walk test (6MWT) with pulse oximetry

1.1.4. Safety Objectives

Evaluate the safety and tolerability of multiple doses of ISIS 702843 in patients with β-Thalassemia Intermedia.

Evaluate the impact of multiple doses of ISIS 702843 on the emergence and/or progression of recognized complications of β-Thalassemia Intermedia.

1.1.5. Pharmacokinetic Objectives

Evaluate pharmacokinetic (PK) exposure over time and potential PK / pharmacodynamic (PD) correlation on relevant biomarkers and efficacy outcome measures.

1.2. Study Endpoints

1.2.1. Primary Endpoint

Proportion of patients who have a ≥ 1.0 g/dL increase from Baseline in Hb, comparing the 3 cohorts at Week 27 of treatment.

- Baseline Hb for Analysis is the mean of all Hb measurements taken in the Screening Period and Day 1, pre-dose. Each measurement used for the calculation of Baseline must be taken at least 6 weeks after the most recent transfusion for that patient. Baseline must be based on at least 2 Hb measurements.
- The value to use for Week 27 of treatment is the mean of the 2 Hb measurements intended to be taken at Day 169 (Week 25) and Day 183 (Week 27); if one of these measurements is missing, then the other measurement will be assigned as the value to use for Week 27 of treatment. For a Hb measurement to be usable for the determination of the value to use for Week 27 of treatment, the sampling must occur at least 6 weeks after the most recent transfusion for that patient.

1.2.2. Secondary Endpoints

Proportion of patients who have a ≥ 1.5 g/dL increase from Baseline in Hb, comparing the 3 cohorts at Week 53 of treatment.

- Baseline Hb for Analysis is as defined above for the Primary Endpoint
- The value to use for Week 53 of treatment is the mean of the 2 Hb measurements intended to be taken at Day 309 (Week 45) and Day 365 (Week 53); if one of these measurements is missing, then the other measurement will be assigned as the value to use for Week 53 of treatment. For a Hb measurement to be usable for the determination of the value to use for Week 53 of treatment, the sampling must occur at least 6 weeks after the most recent transfusion for that patient.

Proportion of patients who have a ≥ 1.0 mg Fe/g dry weight decrease from Baseline in LIC, comparing the 3 cohorts at Week 53 of treatment.

- Baseline LIC is the LIC measurement taken during the Screening Period
- The Week 53 value is the LIC measurement intended to be taken at Day 365 (Week 53)

1.2.3. Exploratory Endpoints

Impact of ISIS 702843 on IE:

- Mean change from Baseline in plasma erythroferrone concentration, comparing the 3 cohorts over time
- Mean change from Baseline in plasma sTfR1 concentration, comparing the 3 cohorts over time
- Mean change from Baseline in reticulocyte count, comparing the 3 cohorts over time
- Mean change from Baseline in CHr, comparing the 3 cohorts over time
- Mean change from Baseline in plasma EPO concentration, comparing the 3 cohorts over time
- Mean change from Baseline in plasma GDF15 concentration, comparing the 3 cohorts over time
- Mean change from Baseline in erythroblast count, comparing the 3 cohorts over time
- Mean change from Baseline in bone marrow iron concentration, comparing the 3 cohorts over time

Impact of ISIS 702843 on measures of iron overload:

- Mean change from Baseline in LIC, comparing the 3 cohorts over time
- Mean change from Baseline in spleen iron concentration, comparing the 3 cohorts over time
- Mean change from Baseline in TSAT, comparing the 3 cohorts over time
- Mean change from Baseline in NTBI, comparing the 3 cohorts over time
- Mean change from Baseline in serum ferritin, comparing the 3 cohorts over time
- Mean change from Baseline in chelator usage, comparing the 3 cohorts over time
- Mean change from Baseline in hepcidin, comparing the 3 cohorts over time

Impact of ISIS 702843 on measures of anemia:

- Mean change from Baseline in Hb, comparing the 3 cohorts over time
- Mean change from Baseline in RBC count, comparing the 3 cohorts over time
- Mean change from Baseline in hematocrit, comparing the 3 cohorts over time

- Mean change from Baseline in mean corpuscular volume (MCV), comparing the 3 cohorts over time
- Mean change from Baseline in mean corpuscular Hb, amount (MCH), comparing the 3 cohorts over time
- Mean change from Baseline in mean corpuscular Hb concentration (MCHC), comparing the 3 cohorts over time

Impact of ISIS 702843 on hemolysis:

- Mean change from Baseline in plasma haptoglobin, comparing the 3 cohorts over time
- Mean change from Baseline in plasma protoporphyrin-IX, comparing the 3 cohorts over time
- Mean change from Baseline in plasma bilirubin (indirect), comparing the 3 cohorts over time
- Mean change from Baseline in plasma LDH, comparing the 3 cohorts over time
- Evaluation of peripheral blood smears, comparing the 3 cohorts over time

Impact of ISIS 702843 on coagulation profile:

- Mean change from Baseline in PT, comparing the 3 cohorts over time
- Mean change from Baseline in INR, comparing the 3 cohorts over time
- Mean change from Baseline in aPTT, comparing the 3 cohorts over time
- Mean change from Baseline in antithrombin III, comparing the 3 cohorts over time
- Mean change from Baseline in Protein S, comparing the 3 cohorts over time
- Mean change from Baseline in Protein C, comparing the 3 cohorts over time

Impact of ISIS 702843 on disease severity and quality of life measures:

- Change in the number of transfusions, comparing the 3 cohorts over time
- Change in chelator usage, comparing the 3 cohorts over time
- Mean change from Baseline in NTDT-PRO©, comparing the 3 cohorts over time
- Mean change from Baseline in SF-36, comparing the 3 cohorts over time
- Mean change from Baseline in PGIC, comparing the 3 cohorts over time
- Mean change from Baseline in ISGA, comparing the 3 cohorts over time
- Mean change from Baseline in FACIT-Fatigue, comparing the 3 cohorts over time

Impact of ISIS 702843 on functional status of the patient:

- Mean change from Baseline in 6MWT, comparing the 3 cohorts over time

1.2.4. Safety Endpoints

Adverse events (AEs), vital signs, clinical laboratory tests (serum chemistry, hematology, urinalysis, coagulation panel, thyroid panel), electrocardiogram (ECG).

Measures of extramedullary hematopoiesis, liver function, splenomegaly, osteoporosis, diabetes, pulmonary hypertension (PHT), and leg ulcers.

1.2.5. PK Endpoints

A PK profile (including pre-dose sampling) over a 6-hour period will be collected at Day 1 (Week 1). Two (2) samples – pre-dose and 3-hour post-dose – will be evaluated at Day 197 (Week 29). Trough levels will be evaluated just prior to each dose through Day 169 (Week 25), as well as on a more limited basis throughout the rest of the Treatment Period. One (1) sample will be evaluated on Day 183 (Week 27); there is no dose at this visit. In the Post-Treatment Period (PTP), 1 sample at each of the 3 planned visits will be evaluated.

2. BACKGROUND AND RATIONALE

2.1. Overview of Disease

β-Thalassemia is an autosomal recessive disorder characterized by abnormal β-globin production. The clinical presentation is highly variable with mild to severe hypochromic microcytic anemia, progressive splenomegaly, and growth retardation. With time, secondary iron overload may evolve with a multitude of symptoms related to excessive tissue iron deposition including liver disease, osteoporosis, diabetes, hypothyroidism, and hypogonadism. Further complications include PHT, heart failure, thrombotic events (e.g., deep vein thrombosis [DVT], stroke), and leg ulcers. Erythroid marrow expansion can cause extramedullary hematopoiesis with hepatosplenomegaly and extramedullary haematopoietic pseudotumors. Excessive hemolysis can result in gallstones.

While thalassemia is an ultra-rare disorder in the USA, it is most common among people of Italian, Greek, Middle Eastern, South Asian, and North African descent. Males and females have similar predilection for the disease.

β-Thalassemia is characterized by IE, anemia, and hemolysis. The etiology of the anemia is multifactorial. Adult Hb comprises 2 α-globin and 2 β-globin ($\alpha 2\beta 2$) chains. In β-Thalassemia, a quantitative deficiency in β-globin results in impaired Hb production with subsequent anemia. Furthermore, due to β-globin deficiency, there is an α-globin chain excess which aggregate to form toxic hemichromes. These hemichromes mediate terminal erythroid maturation arrest and this impaired red cell differentiation further exacerbates the underlying anemia phenotype. In addition, hemichromes along with excessive reactive oxygen species destabilize the red cell membrane rendering them susceptible to hemolysis.

The IE and anemia result in massive expansion of the red cell precursor pool with high demand for iron to support the ineffective production of red cells. To meet this demand, massive intestinal iron hyperabsorption occurs due to suppression of hepcidin, a 25-amino acid hepatic-derived peptide hormone central to iron homeostasis.

Hepcidin serves to limit plasma iron mobilization by binding to ferroportin thereby mediating its internalization and subsequent degradation. Ferroportin is the sole exporter of iron from cells under physiological conditions, and is expressed predominately in the liver, intestinal enterocyte (basal surface), and macrophages. In the presence of iron overload, hepcidin is induced with subsequent destruction of ferroportin thereby suppressing intestinal iron uptake. β-Thalassemia, however, is characterized by aberrant *suppression* of hepcidin despite iron overload (due to excess production of erythroferrone), and hence ferroportin cell surface expression is maintained resulting in continual intestinal iron hyperabsorption and tissue iron overload.

The distinction between the various phenotypes of β-Thalassemia relies primarily on the clinical severity of the disease. Originally β-Thalassemia intermedia was used to describe patients who had clinical manifestations that were too severe to be termed β-Thalassemia minor, yet were too mild to be termed β-Thalassemia Major (Sturgeon et al. 1955). Today, the degree of transfusion dependency further helps differentiate Intermedia from Major, with Intermedia usually not being transfusion dependent (NTDT), and Major characterized by transfusion dependency (TDT). Patients with β-Thalassemia intermedia usually present in later childhood or even adulthood, with mild to moderate anemia and a Hb between 7 and 10 g/dL, which typically is sustainable without the need for regular transfusion therapy.

Multiple modalities of intervention are utilized in the management of β-Thalassemia, depending on the severity of the condition, including blood transfusions, iron chelation therapy, splenectomy, hydroxyurea, and other supportive interventions. Despite numerous therapies, treatments do not alter the progressive course of the disease and there remains a substantial unmet medical need for better disease modifying therapies for β-Thalassemia.

2.2. Therapeutic Rationale

Transmembrane protease, serine 6 (TMPRSS6) is a transmembrane serine protease predominantly expressed in liver cells and is a key negative regulator of hepcidin expression. TMPRSS6 activity suppresses the expression of hepcidin. Therefore, TMPRSS6 inhibition attenuates negative regulation of hepcidin resulting in increased hepcidin expression.

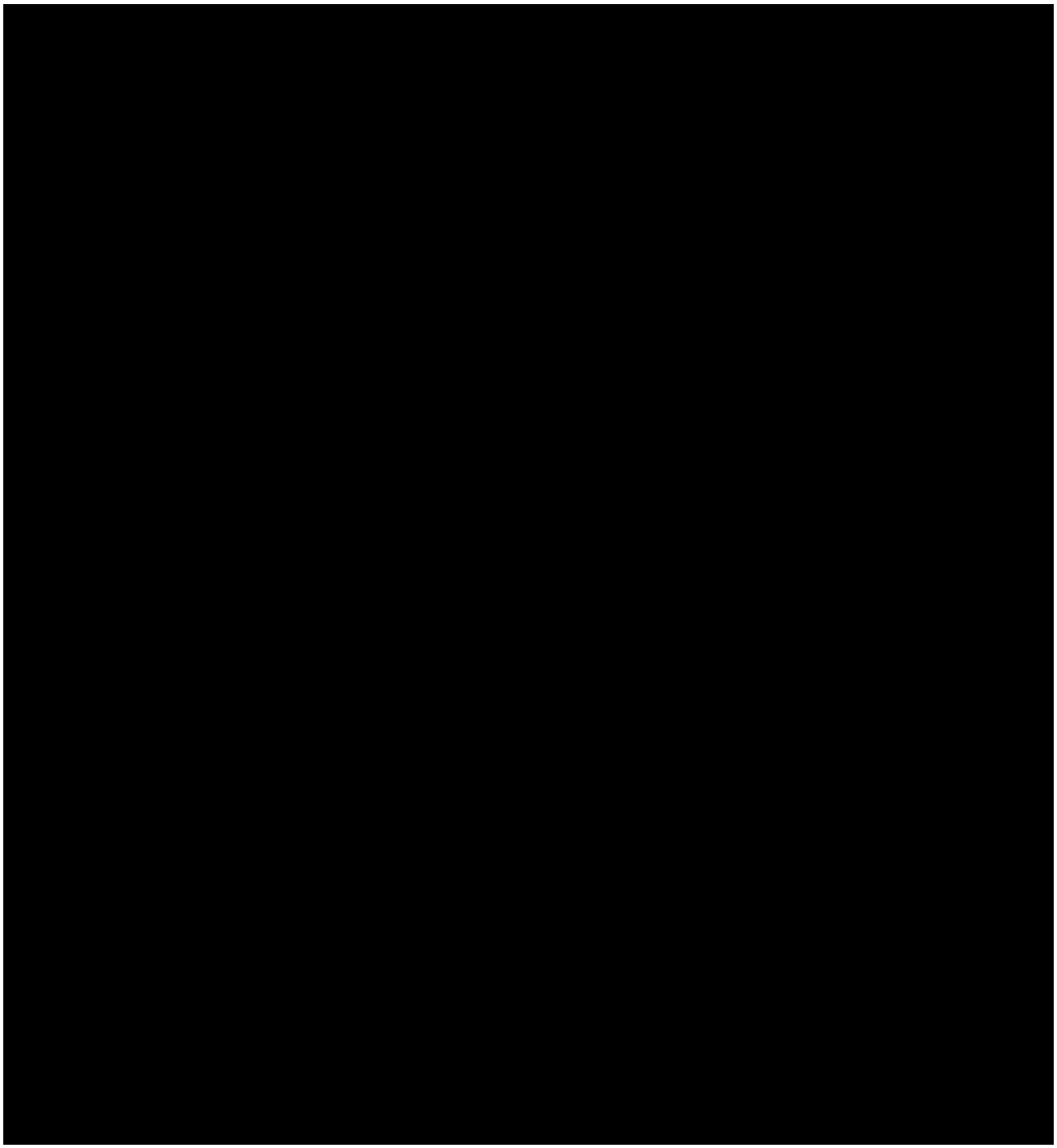
β-Thalassemia is a disease characterized by inappropriately low levels of hepcidin. There is strong evidence from *in vivo* models of β-Thalassemia that TMPRSS6 suppression by raising hepcidin expression may be an effective therapeutic strategy to counter the hypo-hepcidinemic state of β-Thalassemia.

This approach is supported by data generated using a mouse-specific Tmprss6 ASO in a mouse model of β-thalassemia intermedia where reduction of Tmprss6 with Tmprss6 ASO resulted in upregulation of hepcidin associated with reduced intestinal iron mobilization, and amelioration of IE, anemia, and iron overload (see the Investigator's Brochure).

Thus, the mode of action of Tmprss6-ASO therapy is to suppress TMPRSS6, a negative regulator of hepcidin expression, resulting in substantial hepcidin induction. This results in a unique dual action, first impairing intestinal iron uptake resulting in reduced total body iron, and secondly, a substantial improvement in hematological parameters due to less erythroid toxicity manifesting as improved anemia, IE, and hemolytic parameters.

Furthermore, ISIS 702843 has been evaluated in healthy volunteers in a Phase 1 study where dose-dependent increases in serum hepcidin levels and decreases in serum TSAT and serum iron levels were observed following administration of ISIS 702843 (see the Investigator's Brochure), providing initial human pharmacological evidence that TMPRSS6 suppression with ISIS 702843 can effectively induce hepcidin associated with its expected downstream impact on iron metabolism.

2.3. ISIS 702843



[redacted]

2.3.3. Preclinical Experience

Detailed information concerning the preclinical studies conducted with ISIS 702843 can be found in the Investigator's Brochure.

2.3.4. Clinical Experience

Detailed information concerning the clinical studies conducted with ISIS 702843 can be found in the Investigator's Brochure. A summary is included below.

The safety and tolerability of ISIS 702843 was evaluated in a total of 36 healthy volunteer subjects aged 18-64 in a blinded, placebo-controlled, Phase 1 study ISIS 702843-CS1. Of these 36 subjects, 27 received ISIS 702843 and 9 received placebo. ISIS 702843 was well-tolerated at all dose levels evaluated, i.e., up to 60 mg per dose and up to 240 mg total dose (60 mg administered as 4 doses over 8 weeks). ISIS 702843 treatment resulted in decreases in serum iron, TSAT, and reticulocyte Hb, and a dose-dependent increase in hepcidin. There were no dose-dependent, clinically meaningful trends in laboratory assessments, and there were no SAEs.

[redacted] GalNAc conjugation consistently improves the potency of ASOs in humans approximately 20- to 30-fold, and no effects on platelet, renal, or hepatic parameters have been identified from assessment of integrated laboratory test data across all doses tested in Ionis Phase 1 studies of GalNAc-conjugated ASOs in this chemical class (Crooke et al. 2018). Longer-term, Phase 2 data with APO(a)-LRx[redacted] have been published, and no significant changes in platelet count, renal function, or liver function were observed, and neither was a between-group difference in the risk of influenza-like symptoms; the most common adverse events among patients who received APO(a)-LRx were injection site reactions, which were generally mild (Tsimikas et al. 2020).

[redacted]

2.5. Benefit-Risk Assessment

Detailed information concerning the benefit-risk assessment of ISIS 702843 can be found in the Investigator's Brochure.

2.5.1. Benefit Assessment

While ISIS 702843 has been administered to healthy volunteers, it has not been administered to patients with β-thalassemia intermedia. In such patients, TMPRSS6 is a critical negative regulator of hepcidin expression.

ISIS 702843 inhibits TMPRSS6 protein production which, in turn, upregulates hepcidin. Increased production of hepcidin is anticipated to have therapeutic benefit in patients with chronic IE, anemia, and iron overload that is driven by inappropriately low levels of hepcidin with intestinal iron hyperabsorption.

2.5.2. Risk Assessment

Known potential risks to patients in the study include both disease-specific risks and those known potential risks associated with the usage of ASOs.

Disease-specific risks include:

1. Potential exacerbation of anemia due to iron-restricted erythropoiesis with ISIS 702843 usage

   To mitigate this risk, temporary stopping rules have been established to be implemented in the event of a confirmed Hb result < 6.0 g/dL that also represents a decrease from Baseline of at least 1.0 g/dL, or if in the judgment of the Investigator the patient is clinically symptomatic (Section 8.6.1).

2. Excessive Hb elevation
3. To mitigate this risk, temporary stopping rules have been established to be implemented in the event of either (i) a confirmed Hb result ≥ 12.5 g/dL, or (ii) a confirmed Hb increase of ≥ 2.0 g/dL within a 4-week period and Hb > 10.5 g/dL (Section 8.6.1).
4. Disease-related exacerbation of anemia due to intercurrent infection or disease progression

   Mitigation strategies include: Exclusion of advanced severe patients and allowing standard-of-care interventions to aggressively treat all disease exacerbations.
5. Thalassemia intermedia-related safety concerns/morbidities due to increasing severity and chronicity of the disease

   These include: thrombosis, splenic rupture, PHT, leg ulcers, diabetes, abnormal liver function, and heart failure. Mitigation strategies include: Exclusion or monitoring for the above safety concerns and early intervention as per standard of care.

Potential risks associated with the usage of ASOs include:

1. AEs at the injection site

   These should be managed by standard treatment of AEs at the injection site.
2. Thrombocytopenia

   Mitigation strategies include: Exclusion of patients with history of coagulopathy / immune thrombocytopenia, regular platelet monitoring, and implementation of well-defined platelet stopping rules.
3. Hepatotoxicity

   Mitigation strategies include: Regular monitoring of liver function tests (LFTs) and implementation of well-defined LFT-related stopping rules.
4. Nephrotoxicity

   Mitigation strategies include: Exclusion of patients with impaired renal function, i.e., estimated glomerular filtration rate (eGFR) < 45 mL/min/1.73 m^2 [using Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation] or urine protein-to-creatinine ratio (UPCR) ≥ 0.50 mg/mg, regular monitoring of renal function, and implementation of well-defined renal function test-related stopping rules.

2.5.3. Overall Assessment of Benefit:Risk

Taking into account the measures taken to minimize risk to patients participating in this study, the potential risks identified in association with ISIS 702843 are justified by the anticipated benefits that may be afforded to patients with non-transfusion dependent β-thalassemia intermedia for which there are currently no known effective disease-modifying therapies.

2.5.4. Additional Risks During the COVID-19 Pandemic

During the Coronavirus Disease 2019 (COVID-19) pandemic, patients with β-thalassemia intermedia face additional risks based on underlying comorbidities stemming from IE, chronic

hemolytic anemia, and iron overload; thus, β-thalassemia intermedia patients may be more severely affected by COVID-19 relative to the general population (Taher et al. 2020).

At the present time, preliminary reports are available regarding the COVID-19 mortality risk for non-transfusion dependent β-thalassemia patients versus for the general population. (Karimi et al. 2020) reported that in Iran the COVID-19 mortality rate is 26.1% (6/23) for β-thalassemia patients, compared to 6.34% for the general population. However, (Motta et al. 2020) reported that there were no deaths among 11 Italian β-thalassemia patients (one of which had β-thalassemia intermedia) known to have been infected with the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes COVID-19, suggesting no risk of increased severity of COVID-19 in these patients. A larger number of cases needs to be evaluated before a clear conclusion can be drawn with respect to COVID-19 mortality rate in non-transfusion dependent β-thalassemia patients.

To maintain the favorable benefit:risk ratio of ISIS 702843 for non-transfusion dependent β-thalassemia intermedia patients (Section 2.5.3), additional measures will be taken during a coronavirus disease pandemic to reduce the likelihood that patients participating in this study will become infected with the coronavirus that causes the disease. The mitigation strategies are provided in Section 3.8 – Allowances in the Circumstance of a Public Health Emergency. These measures (e.g., replacing scheduled clinic visits with remote assessments or at-home visits or a combination) are to be implemented at the discretion of the PI.

3. EXPERIMENTAL PLAN

3.1. Study Design

ISIS 702843-CS2 is a multi-center, randomized, open-label study of SC administered ISIS 702843 in patients with non-transfusion dependent β-Thalassemia Intermedia.

The study will comprise 3 cohorts – Cohorts A, B, and C. However, upon Amendment 2 approval, (i) the dose level for Cohorts A and B will become the same at 120 mg ISIS 702843, and (ii) no new patients will be randomized to Cohort A. There will be approximately 12 eligible patients in Cohorts A and B combined, and approximately 12 eligible patients in Cohort C, for a total of approximately 24 eligible patients overall. These patients will be stratified based on Screening Mean Hb for Eligibility (defined in Section 6.1.1), low (≤ 8.0 g/dL) and high (> 8.0 g/dL). Then patients will be randomized in parallel to Cohort B (120 mg ISIS 702843 per dose) or Cohort C (120 mg ISIS 702843 per dose for three consecutive doses, followed by 160 mg ISIS 702843 per dose, if dose escalation is allowed based on a demonstration of adequate safety).

Dose escalation to 160 mg ISIS 702843 in Cohort C will be done on an individual patient basis, and only after clinical and laboratory safety evaluations related to the prior dose (scheduled to be 28 days prior) have been completed, and there is an acceptable safety profile as determined by the Investigator and the Sponsor Medical Monitor. Safety data to be reviewed include AEs and concomitant medications. For dose escalation to be considered, a patient must not have met a temporary or permanent stopping rule (Section 8.6) at any time since their prior 3 consecutive doses (scheduled to be 28, 56, and 84 days prior).

In addition, ISIS 702843 dose adjustments (decreases and increases to the proposed maximum clinical dose of 160 mg per 28-day interval) are permitted in all three cohorts, as described in Section 8.7.

Each patient will be treated for up to 2 years, receiving up to 27 doses of ISIS 702843, with a planned 28-day interval between each dose.

This is an open-label study. Nevertheless, to facilitate obtaining objective feedback from the patients on their quality of life measures, it is preferable that patients not be informed of how their dose compares to the dosing levels of other cohorts and individual patients.

3.2. Number of Study Centers

This study will be conducted at multiple centers worldwide, including locations planned in Southeast Asia, Australia, and Mediterranean countries.

3.3. Number of Patients

The study will enroll approximately 24 evaluable patients overall, with approximately 12 evaluable patients in Cohorts A and B combined, and approximately 12 evaluable patients in Cohort C. Patients who terminate early from the study may be replaced, with a maximum of 3 replacements for Cohorts A and B combined, and with a maximum of 3 replacements for Cohort C. Patients who discontinue after Day 365 (Week 53) will not be replaced. Each additional patient will be assigned to the same cohort and stratum (i.e., Hb $\leq$ 8.0 g/dL, or Hb > 8.0 g/dL) as the patient they are replacing. Before a fit is identified, patients who do not qualify for that stratum will still be enrolled in the study, including into that cohort.

The maximum enrollment for the study will be limited to 30 patients or until enrollment is closed by the Sponsor. The Sponsor may close enrollment to one cohort before another; for example, it is likely that enrollment to Cohort B will be closed before enrollment to Cohort C because the intent is to have approximately 12 evaluable patients in Cohorts A and B combined, and approximately 12 evaluable patients in Cohort C.

3.4. Overall Study Duration and Follow-up

The study will consist of Screening, Treatment, and Post-Treatment periods. Please refer to the Schedule of Procedures in Appendix A.

The length of each patient's participation in the study is approximately 29 months that includes an approximately 2-month Screening Period, a 24-month Treatment Period, and a 3-month Post-Treatment Period (PTP).

Patients who discontinue from ISIS 702843 treatment and agree to remain in the study will attend the following clinic visits: Early Termination from Treatment (ETFT), PTP Visit 1, PTP Visit 2, and PTP Last Visit. If the patient is unable or unwilling to participate in all of these visits, the PI should determine the follow-up procedures the patient would agree to complete. Every effort should be made to complete the ETFT procedures and observations at the time of withdrawal.

Patients may be required to attend additional visits for monitoring of AEs or abnormal investigation results. The frequency of additional monitoring will be determined by the Investigator in consultation with the Medical Monitor.

3.4.1. Screening

Patient eligibility for the study will be determined within 8 weeks prior to study entry. Four Screening Visits (SVs) are planned – SV1, SV2, SV3, and SV4:

- SV1 supports all initial Screening evaluations
- SV2 supports additional Hematology testing related to the evaluation of Inclusion Criterion 5, and the collection of additional information related to the following measures of disease severity, quality of life, and functional status of the patient: NTDT-PRO©, SF-36, PGIC, ISGA, FACIT-Fatigue, and 6MWT with pulse oximetry
- SV3 supports additional Hematology testing related to the evaluation of Inclusion Criterion 5, and analysis of Chemistry Panel, Urinalysis, and Coagulation Panel samples to provide results for these parameters closer to Day 1 than is SV1. No Clinic Visit is scheduled for SV3; blood and urine samples may be collected at Study Clinic, at a local phlebotomy clinic, or by a home healthcare provider, at PI discretion. The testing should be done at the central laboratory except when Section 3.8 applies, in which case a local laboratory is acceptable provided that the local laboratory results are recorded by the site into the electronic database.
- SV4 supports additional Hematology testing related to the evaluation of Inclusion Criterion 5. No Clinic Visit is scheduled for SV4; blood and urine samples may be collected at Study Clinic, at a local phlebotomy clinic, or by a home healthcare provider, at PI discretion. The testing should be done at the central laboratory except when Section 3.8 applies, in which case a local laboratory is acceptable provided that the local laboratory results are recorded by the site into the electronic database.

Magnetic resonance imaging (MRI; needed to evaluate Inclusion Criterion 6) and Ultrasound may be performed anytime within the Screening Period; however, it is preferred for these tests to be performed once the patient has met all other Inclusion/Exclusion criteria, with the exception of Inclusion Criterion 5 because this criterion is evaluated based on all Hb measurements taken in the Screening Period. MRI and Ultrasound should be conducted within 1 week of one another – ideally on the same day.

3.4.2. Treatment

Eligible patients will be stratified based on Screening Mean Hb for Eligibility (defined in Section 6.1.1), low (≤ 8.0 g/dL) and high (> 8.0 g/dL). Then patients will be randomized in parallel to Cohort B (120 mg ISIS 702843 per dose) or Cohort C (120 mg ISIS 702843 per dose for three consecutive doses, followed by 160 mg ISIS 702843 per dose, if dose escalation is allowed based on a demonstration of adequate safety [Section 3.1]). No patients will be randomized to Cohort A after Amendment 2 approval.

Unless the allowances in the circumstance of a public health emergency apply (Section 3.8), through Day 365 (Week 53) these patients will report to the Study Center for each ISIS 702843

administration, with all doses being administered by delegated staff or by trained patient/caregiver under the observation of Study Center staff, and with a planned 28-day interval between each dose. After Day 365 (Week 53) – earlier if Section 3.8 applies – self-administration at home is allowed after appropriate training of patient/caregiver, with training related to the injection step itself encouraged to start as early as Day 1 (e.g., patient or caregiver observes the injection on Day 1 and performs the injection(s) under the observation of Study Center staff at subsequent visits). Thus, planned clinic visits are less frequent after Day 365 (Week 53), despite the planned interval between each dose remaining 28 days throughout the Treatment Period. In the absence of self-administration training, ISIS 702843 will continue to be administered, with the planned interval between each dose remaining 28 days, either at the Study Center by delegated staff or via home visits by Study Center staff or home healthcare providers.

3.4.3. Post-Treatment

Patients are to return to the Study Center for follow-up visits on the following study days from Day 1: 757, 785, and 820. Note that these values for study day from Day 1 are only accurate for those patients who do not discontinue from ISIS 702843 treatment. For this reason, these study days from Day 1 are also referred to as PTP Visit 1, PTP Visit 2, and PTP Last Visit. The final study visit will be PTP Last Visit.

3.5. End-of-Study

The End-of-Study is defined as the date of the last visit of the last patient.

3.6. Internal Safety Committee

An external Data and Safety Monitoring Board will not be utilized for this study.

Data will be reviewed by an internal safety committee. This committee will be comprised of at least 2 physicians who are experienced in the conduct of clinical trials, and are independent of any other elements of the conduct of the study. This committee will be assembled to independently review safety, tolerability, and, as needed, efficacy data collected during this study.

Based on its ongoing assessment of safety, tolerability, and efficacy of ISIS 702843, this committee will provide recommendations to the study team for modifying, stopping, or continuing the study as planned. These decisions will be recorded in the minutes of the meetings. Details on the safety assessments, frequency of review, meeting schedules, and access to data will be outlined in the internal safety committee Charter and Statistical Analysis Plan (SAP).

Safety will be monitored in an ongoing fashion throughout the study by the Medical Monitor and the Drug Safety Physician.

3.7. Dose Adjustments

Patients will be randomized in parallel to 1 of the planned cohorts, with randomization to Cohort A stopping upon Amendment 2 approval (Section 4.2).

ISIS 702843 dose adjustments (increases and decreases) in addition to the dose escalation to 160 mg planned in Cohort C (Section 3.1) will be permitted (Section 8.7). These adjustments may be applicable to one or both of the following: (i) an entire cohort, but only after the first interim analysis; (ii) an individual patient, but only after that individual has completed PK and PD evaluations associated with Day 281 (Week 41).

3.8. Allowances in the Circumstance of a Public Health Emergency

If an investigative site or the clinical trial patients associated with that site experience a public health emergency, such as a pandemic, then throughout that time the following changes to what is written elsewhere in this protocol are permissible, at the discretion of the PI, provided that all ICH, GCP, and regulatory requirements associated with the study are still upheld. These allowances may be implemented at any stage of the study: Screening Period, Treatment Period, and Post-Treatment Period. What is considered a public health emergency is based on circumstances and procedures at the site, and the judgment of the PI. The time at which a public health emergency has resolved to sufficient extent such that these allowances are no longer applicable is based on the judgment of the PI in consultation with the Medical Monitor.

- Remote assessments and at-home visits: Even when an investigative site remains open to clinical trial patients coming on site, social distancing strategies may result in some patients being unwilling or unable to attend protocol-specific clinic visits. For such reasons, scheduled clinic visits may be replaced by remote assessments (via videoconference or telephone call) or at-home visits or a combination, provided that these are properly documented. This may be accomplished by at-home visits by Study Center staff or home healthcare providers, with incorporation of videoconference or telephone assessments by Study Center staff or designee.
- Randomization: When a patient is found to be eligible for the study, the decision regarding if and when the patient will be randomized is based on the judgment of the PI in consultation with the Medical Monitor. Randomization may be delayed by up to 1 month without rescreening.
- Safety assessments: Every effort should be made to continue performing safety assessments on schedule. This may require remote assessments (e.g., for adverse events and concomitant medications) by Study Center staff, and it may require at-home visits (e.g., for blood draws, urine samples, physical examination, vital signs) by Study Center staff or home healthcare providers. Which safety assessments are considered critical, for example to support a decision on whether to administer the next scheduled dose of ISIS 702843 to a patient, is to be determined on a case-by-case basis by the PI in consultation with the Medical Monitor. Critical and noncritical safety assessments that are delayed beyond the protocol-specific visit windows will be considered major and minor, respectively, protocol deviations.
- Reporting of protocol deviations: All protocol deviations that are caused by a public health emergency should be documented as such; for example, include in the description of the protocol deviation that it is related to COVID-19. The impact such protocol deviations had on the study is to be summarized in the clinical study report.

This summary is considered sufficient notification of the EC / IRB for minor protocol deviations that were caused by a public health emergency.

- Laboratory and imaging assessments associated with primary or secondary endpoints: Every effort should be made to perform on schedule Hematology throughout the Screening Period, on Day 1, and at Weeks 27 and 53; and LIC at Screening and at Week 53. For Hematology, this may require at-home visits by Study Center staff or home healthcare providers. Delays to these assessments beyond the protocol-specific visit windows will be considered major protocol deviations. For a given patient, the imaging center and machine used for MRI should not be changed from that used to determine Baseline, and such a change at Week 53 will be considered a major protocol deviation.
- All other laboratory and imaging assessments: Other than critical safety assessments and those assessments that are associated with primary or secondary endpoints (above), laboratory and imaging assessments that are delayed beyond the protocol-specific visit windows will be considered minor protocol deviations. All assessments that are missed will be considered major protocol deviations. For a given patient, it is preferable not to change the imaging center and machine used for DEXA, Echocardiography, and Ultrasound from that used to determine Baseline, and such a change will be considered a minor protocol deviation.
- Evaluations of disease severity and quality of life: PGIC, FACIT-Fatigue, NTDT-PRO©, and SF-36 surveys may be completed during remote assessments (videoconference or telephone) or at-home visits; this may include the patient providing verbal answers that another study-related individual documents. The ISGA survey may be completed at the discretion of the PI. Surveys that are delayed beyond the protocol-specific visit windows will be considered minor protocol deviations. Surveys that are missed will be considered major protocol deviations.
- Evaluations of functional status of the patient: Planned occurrences of the 6MWT with pulse oximetry that are delayed beyond the protocol-specific visit windows will be considered minor protocol deviations. Planned occurrences of the 6MWT with pulse oximetry that are missed will be considered major protocol deviations.
- ISIS 702843 administration: Self-administration is permissible at and before Day 365 (Week 53) after appropriate training of patient/caregiver. To support this eventuality, note that training of patient/caregiver may begin as early as Day 1 (Section 3.4.2). Also permissible is ISIS 702843 administration during an at-home visit by a Study Center staff member or a home healthcare provider, within the scope of their training and qualifications, as appropriate.
  - To support ISIS 702843 administration at home, delivery of study drug to the patient may be undertaken at the discretion of the PI, based on local and regional regulations for transporting investigational product.
- Visit windows: All visit windows will remain as given in Appendix A . Each assessment or event (e.g., ISIS 702843 administration) that occurs outside its corresponding visit window will be considered a protocol deviation. For assessments,

whether such a protocol deviation is to be considered major or minor is discussed earlier in this section. ISIS 702843 administration that occurs outside the visit window will be considered a major protocol deviation.

- Testing at local laboratories: If it is not practical to conduct testing at the central laboratory, then it is permissible to use a local laboratory instead. In these instances, the local laboratory results should be recorded by the site into the electronic database.

4. PATIENT ENROLLMENT

4.1. Screening

Before patients may be enrolled into the study, the Sponsor requires a copy of the written approval of the protocol, informed consent form, and all other patient information and/or recruitment materials from the Study Center Ethics Committee (EC) / Institutional Review Board (IRB).

Patients or their legally acceptable representatives must sign the consent form before any screening tests or assessments, including fasting prior to screening blood draws, are performed. At the time of consent, the patient will be considered enrolled into the study and will be assigned a unique screening number before any study procedures, including Screening procedures, are performed. At the time of randomization, patients will be assigned a unique patient identification number. This number will be used to identify the patient throughout the trial, and must be used on all study documentation related to that patient. The screening number and patient identification number will remain constant throughout the entire trial. Screening numbers and patient identification numbers, once assigned, will not be re-used.

4.2. Randomization

Patients will be randomized on Day 1, after all Screening assessments have been completed and after the Investigator has verified that they are eligible per criteria in Section 5.1 and 5.2. No patient may begin treatment prior to randomization and assignment of a unique patient identification number.

Eligible patients will be randomized using an automated system, such as Interactive Response Technology (IRT). Patients will be stratified based on Screening Mean Hb for Eligibility (defined in Section 6.1.1), low (≤ 8.0 g/dL) and high (> 8.0 g/dL), and then patients will be randomized in parallel to Cohort B (120 mg ISIS 702843 per dose) or Cohort C (120 mg ISIS 702843 per dose for 3 consecutive doses, followed by 160 mg ISIS 702843 per dose, if dose escalation is allowed based on a demonstration of adequate safety [Section 3.1]), following a prespecified randomization schema. No patients will be randomized to Cohort A after Amendment 2 approval. The Sponsor's designee will prepare the randomization list.

4.3. Replacement of Patients

Patients who terminate early from the study may be replaced, with a maximum of 3 replacements for Cohorts A and B combined, and with a maximum of 3 replacements for Cohort C. Patients who discontinue after Day 365 (Week 53) will not be replaced. Each additional patient will be

assigned to the same cohort and stratum (i.e., Hb ≤ 8.0 g/dL, or Hb > 8.0 g/dL) as the patient they are replacing. Before a fit is identified, patients who do not qualify for that stratum will still be enrolled in the study, including into that cohort.

The maximum enrollment for the study will be limited to 30 patients or until enrollment is closed by the Sponsor. The Sponsor may close enrollment to one cohort before another; for example, it is likely that enrollment to Cohort B will be closed before enrollment to Cohort C because the intent is to have approximately 12 evaluable patients in Cohorts A and B combined, and approximately 12 evaluable patients in Cohort C.

4.4. Treatment Assignment

This is an open-label study. Nevertheless, to facilitate obtaining objective feedback from the patients on their quality of life measures, it is preferable that patients not be informed of how their dose compares to the dosing levels of other cohorts and individual patients.

5. PATIENT ELIGIBILITY

To be eligible to participate in this study, candidates must meet the following eligibility criteria within 8 weeks of Study Day 1. The Medical Monitor may be consulted if any questions arise regarding Inclusion or Exclusion Criteria listed below. The mean of all values obtained during the Screening Period is generally considered sufficient when making a decision regarding eligibility; however, the Medical Monitor will also consider trends in the data and the significance of one or more individual values that are outside of acceptable limits when making a determination.

5.1. Inclusion Criteria

1. Patient must have given written informed consent and be able to comply with all study requirements
2. Aged 18-65 years old, inclusive, at the time of informed consent
3. Clinical diagnosis of β-Thalassemia Intermedia with genotypic confirmation of β-globin gene mutations including but not limited to Hemoglobin E (HbE)/β-thalassemia
4. Patient must be non-transfusion dependent as defined by: No more than 8 transfusions in the past 12-month period, and no transfusions in the 8-week period prior to Day 1
5. Mean Hb within the range 6.0–10.5 g/dL, inclusive, with this mean based on all Hb measurements taken in the Screening Period that are at least 6 weeks after the most recent transfusion for that patient. This mean must be based on at least two Hb measurements.
6. LIC within the range of 3.0–20.0 mg Fe/g dry weight, inclusive
7. Chelators will be permitted provided the dose has not been increased for at least 2 months prior to Day 1, with LIC > 5.0 mg Fe/g dry weight and serum ferritin > 300 ng/mL
8. Females must be non-pregnant and non-lactating, one of the following: (i) surgically sterile (e.g., tubal occlusion, hysterectomy, bilateral salpingectomy, bilateral

oophorectomy), (ii) postmenopausal (defined as 12 months of spontaneous amenorrhea without an alternative medical cause and follicle stimulating hormone [FSH] levels in the postmenopausal range for the laboratory involved), (iii) abstinent*, or (iv) if engaged in sexual relations and of child-bearing potential, the patient must be using a highly effective (Section 6.3.1) contraceptive method from the time of signing the informed consent until at least 13 weeks after the last dose of ISIS 702843.

Males must be one of the following: (i) surgically sterile, (ii) abstinent*, or (iii) if engaged in sexual relations with a female of child-bearing potential, the patient must be using a highly effective (Section 6.3.1) contraceptive method from the time of signing the informed consent until at least 13 weeks after the last dose of ISIS 702843.

* Abstinence is only acceptable as true abstinence, i.e., when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a trial, and withdrawal are not acceptable methods of contraception.

5.2. Exclusion Criteria

1. Genotypic confirmation of either α-globin gene triplication or sickle hemoglobin (HbS)/β-thalassemia, as determined by genetic assessment of a panel of blood-related disorders
2. Clinically significant abnormalities in medical history or physical examination, which at the discretion of the PI will pose significant additional risk to the patient in participating in the study
3. Clinically significant abnormalities in Screening laboratory values that would render a patient unsuitable for inclusion, at the discretion of the PI
4. Current use of iron-chelation therapy if LIC is 3.0–5.0 mg Fe/g dry weight, inclusive, or if serum ferritin ≤ 300 ng/mL
5. Symptomatic splenomegaly, including abdominal pain or organ obstruction, or evidence of hypersplenism, such as low white blood cell (WBC) count and/or low platelets
6. Platelet count Screening laboratory results < 120,000/mm^3 or > 1,000,000/mm^3, or any other clinically significant abnormalities in platelet count Screening laboratory values that would render a patient unsuitable for inclusion
7. Significant concurrent/recent coagulopathy; history of non-traumatic significant bleeding; history of immune thrombocytopenic purpura (ITP); current use of SC anti-coagulants; history of thrombotic events, including stroke or DVT
8. Clinically significant renal dysfunction which at the discretion of the PI will pose significant additional risk to the patient in participating in the study
9. Either of the following confirmed manifestations of renal dysfunction:

    9a. eGFR < 45 mL/min/1.73 m^2, using CKD-EPI equation

    9b. Proteinuria manifesting as UPCR ≥ 0.50 mg/mg

10. Clinically significant liver function test (LFT) abnormalities at the discretion of the PI
11. Alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) > 3.0 × ULN
12. Historical diagnosis of cirrhosis, or current signs and symptoms of cirrhosis
13. Fasting blood glucose > 2.0 × ULN
14. Significant PHT defined as tricuspid regurgitation > 3.0 meters per second (m/s) on echocardiography and/or requiring treatment
15. Uncontrolled hypertension (which for this protocol is considered > 140 mm Hg systolic or > 90 mm Hg diastolic)
16. Heart failure class 3 or higher (New York Heart Association, NYHA)
17. Ejection fraction < 50% by echocardiogram, multigated acquisition (MUGA), or cardiac magnetic resonance imaging (MRI)
18. Patients unable to have MRI performed, for example, because of a pacemaker or implantable cardioverter-defibrillator. (MRI is being used to measure LIC.)
19. Active infection requiring systemic antiviral or antimicrobial therapy that will not be completed prior to Day 1
20. Active infection with human immunodeficiency virus (HIV), hepatitis C, or hepatitis B diagnosed by initial serological testing and confirmed with RNA testing, or prior treatment for hepatitis C. Patients at Screening who test positive by serology, but negative by RNA may be allowed in consultation with the Sponsor Medical Monitor
21. Unwillingness to comply with study procedures, including follow-up, as specified by this protocol, or unwillingness to cooperate fully with the Investigator
22. Recent introduction of hydroxyurea (6 months prior to Day 1)
23. Treatment with or exposure to another investigational drug, biological agent, ASO, small interfering ribonucleic acid (siRNA), or device within 1 month of Screening, or 5 half-lives of investigational agent, whichever is longer; or:
    - Treatment with or exposure to sotatercept (ACE-011), luspatercept-aamt (Reblozyl®), or ruxolitinib (Jakafi®) within 4 months of Screening
    - Treatment with or exposure to hematopoietic stimulating agents (e.g., EPOs) or any hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs) (e.g., roxadustat, vadadustat, daprodustat, molidustat, desidustat) within 8 weeks of Day 1
    - Prior bone marrow transplant, stem cell transplant, or gene therapy
24. Regular use of alcohol within 6 months prior to Screening [> 7 drinks/wk for females, > 14 drinks/wk for males (1 drink = 5 ounces [150 mL] of wine or 12 ounces [360 mL] of beer or 1.5 ounces [45 mL] of hard liquor)]
25. Surgery associated with significant blood loss within 4 months of Screening, splenectomy within 12 months of Screening, or splenectomy scheduled during the Treatment Period

26. Use of iron supplements, including iron-containing vitamins, within 4 months of Screening
27. Pregnant or lactating
28. Have any other conditions which, in the opinion of the PI, would make the patient unsuitable for inclusion, or could interfere with the patient participating in or completing the study
29. Active infection with the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that causes COVID-19. (Note: It is acceptable to receive COVID-19 vaccinations while in this study, including in the Screening Period, but it is preferred that there is an interval of at least 7-10 days between each vaccination and ISIS 702843 dose.)

6. STUDY PROCEDURES

6.1. Study Schedule

All required study procedures are outlined in Appendices A–C. The safety of ISIS 702843 will be monitored in an ongoing fashion throughout the study by the Investigators, the Medical Monitor, the Drug Safety Physician, and an internal safety committee (Section 3.6).

6.1.1. Screening and Baseline

Written informed consent for the study will be obtained prior to the performance of any study-related procedures, including screening procedures and fasting prior to screening blood draws. An 8-week period, during which 4 screening visits are planned (Section 3.4.1), is provided for completing screening assessments and determining patient eligibility for the study. Safety labs may be retested for determination of patient eligibility after consultation with the Medical Monitor.

During the Screening Period:

- Patients will undergo a medical history and physical examination including vital signs, 12-lead ECG, and have blood and urine samples taken for clinical laboratory testing, including chemistry panel, iron metabolism panel, hematology, coagulation, FSH, thyroid panel, and urinalysis
- A blood sample will be taken for genotyping of β-globin variants (related to Inclusion Criterion 3) and a genetic assessment of a panel of blood-related disorders that will include determination of α-globin copy number and whether the patient has exclusionary sickle hemoglobin (HbS)/β-thalassemia (related to Exclusion Criterion 1)
- The patient's transfusion history will be documented, taking into account, at a minimum, all blood transfusions performed in the past 12-month period; a longer history over the previous 3 years or more is preferred

- The patient's history regarding use of chelation will be documented, taking into account, at a minimum, all chelator usage in the past 12-month period; a longer history over the previous 3 years or more is preferred
- MRI will be performed to assess LIC, spleen iron concentration, and, if feasible, bone marrow iron concentration, as well as liver and spleen volume. Ultrasound will be performed to assess liver and spleen size. MRI and Ultrasound may be carried out anytime within the Screening Period; however, it is preferred for these evaluations to be performed once the patient has met all other Inclusion/Exclusion criteria, with the exception of Inclusion Criterion 5 because this criterion is evaluated based on all Hb measurements taken in the Screening Period, as described below (Screening Mean Hb for Eligibility). In the Screening Period and at all applicable time points in the Treatment Period, MRI and Ultrasound should be conducted within 1 week of one another – ideally on the same day.
- Additional information will be collected related to the following measures of disease severity, quality of life, and functional status of the patient: NTDT-PRO©, SF-36, PGIC, ISGA, FACIT-Fatigue, and 6MWT with pulse oximetry. This information will be collected twice during the Screening Period: at SV1 and SV2.

Screening Mean Hb for Eligibility

Screening Mean Hb for Eligibility is determined for the purposes of eligibility, and is based on all Hb measurements that were taken in the Screening Period, and this mean is used to evaluate Inclusion Criterion 5. Each Hb measurement used for the calculation of this mean must be taken at least 6 weeks after the most recent transfusion for that patient. This mean must be based on at least 2 Hb measurements.

Baseline Hb for Analysis

Baseline Hb for Analysis is defined as the mean of all Hb measurements that were taken in the Screening Period and Day 1, pre-dose. Each Hb measurement used for the calculation of this mean must be taken at least 6 weeks after the most recent transfusion for that patient. This mean must be based on at least 2 Hb measurements (See Section 10.4).

Baseline for all Other Attributes

Baseline for all other attributes is defined in Section 10.4.

6.1.2. Treatment Period

During the 2-year Treatment Period, each patient will receive up to 27 SC doses of ISIS 702843, with a planned 28-day interval between each dose (i.e., Day 1 [Week 1], Day 29 [Week 5], Day 57 [Week 9], …, Day 729 [Week 105]). Unless the allowances in the circumstance of a public health emergency apply (Section 3.8), through Day 365 (Week 53) these patients will report to the Study Center for each ISIS 702843 administration, with all doses being administered by delegated staff or by trained patient/caregiver under the observation of Study Center staff. After Day 365 (Week 53) – earlier if Section 3.8 applies – self-administration at home is allowed after appropriate training of patient/caregiver.

Safety and clinical laboratory evaluations, as well as blood sampling for PK analysis, will be performed periodically throughout the Treatment Period (Appendices A–C). Any AEs and concomitant medications will be recorded. All safety data, including AEs and concomitant medications, will be reviewed by the Medical Monitor and the Drug Safety Physician on an ongoing basis throughout the study.

Patients who discontinue from ISIS 702843 treatment and agree to remain in the study will attend the following clinic visits: ETFT, PTP Visit 1, PTP Visit 2, and PTP Last Visit. If the patient is unable or unwilling to participate in all of these visits, the PI should determine the follow-up procedures the patient would agree to complete. Every effort should be made to complete the ETFT procedures and observations at the time of withdrawal.

6.1.3. Post-Treatment Period

After the last dose, scheduled for Day 729 (Week 105) – but will occur earlier for early termination patients – patients will return to the clinic for safety assessments at 4- to 5-week intervals, for a total of 3 PTP visits spanning 13 weeks.

Safety and clinical laboratory evaluations, as well as blood sampling for PK analysis, will be performed periodically throughout the PTP (Appendix A–Appendix C). Any AEs and concomitant medications will be recorded. All safety data, including AEs and concomitant medications, will be reviewed by the Medical Monitor and the Drug Safety Physician on an ongoing basis throughout the study.

6.2. Study Assessments

6.2.1. Physical Exams and Vital Signs

Physical exams and vital signs will be performed as indicated in the Schedule of Procedures (Appendix A). Vital signs include weight (at specific visits), blood pressure (BP), heart rate, respiratory rate, and body temperature. Blood pressure and heart rate will be recorded after the patient has been in a sitting (semi-supine) position for at least 5 minutes, using the same arm (preferentially the left arm). Height will be measured at Screening.

6.2.1.1. Leg Ulcers

An evaluation for the presence of leg ulcers will be part of each physical examination - not only the full physical examination to be performed at Screening but also the abbreviated physical examination to be conducted at the times indicated in Appendix A, to assess changes from Screening. Leg ulcers will be measured (shortest and longest axes) and documented via photographs taken from a consistent distance and with a ruler next to the ulcer for reference of length.

6.2.2. Laboratory and Imaging Assessments

Laboratory analyte samples will be collected throughout the study as indicated in Appendix A. A list of these analytes is contained in Appendix B. Blood chemistry should be taken after fasting for approximately 8-10 hours. During this time, the patient can drink water and they should ensure that they consume sufficient water in order to not become dehydrated. Due to the

length of time between visits, a reminder should be communicated to the patient prior to each visit where a fasting blood sample is collected.

Investigators may, at their discretion, test specific laboratory parameters at their local laboratory for safety assessments prior to dosing. In these instances, the local laboratory results should be recorded by the site into the electronic database.

Sampling time for hepcidin testing is preferably at the same time of day from one occurrence to the next for a given patient; ideally, this consistent sampling time will be in the morning.

PK blood sampling will occur prior to dosing at several time-points throughout the Treatment Period as well as post-dose on Day 1 and Day 197 (Week 29). This will require patients to remain at the clinic for approximately 6 hours (Day 1) and 3 hours (Day 197 [Week 29]) after ISIS 702843 dosing – see Appendix C.

If the platelet value, serum creatinine, or liver enzyme tests are uninterpretable (e.g., due to clumping, hemolysis, or quantity not sufficient) or missing, then a repeat blood specimen should be re-drawn as soon as possible – ideally within 7 days, should be within 2 weeks – which may require an unscheduled visit or blood draw.

Investigators may, at their discretion, test specific laboratory parameters that may be prone to clotting, clumping, or hemolysis (e.g., hematology samples) at their local laboratory, in addition to the required central laboratory samples. In these instances, the local laboratory results should be recorded by the site into the electronic database.

Platelet count will be monitored as described in Section 8.5.2. The Investigator should review all platelet count results within 48 hours of receipt. In the event of any platelet count $< 100{,}000/mm^3$, monitoring frequency and dosing should be adjusted as recommended in Section 8.6.6. In the event of an unreportable platelet count result (e.g., due to a hemolyzed or clumped blood sample), patient dosing cannot continue until another sample is tested (at a central or local laboratory) and determined not to have met a stopping rule.

Any case of a platelet count $< 50{,}000/mm^3$ should be reported to the Sponsor in an expedited fashion (see Section 8.6.6). The Medical Monitor will discuss the assessment and treatment of such patients with the PI, considering whether any additional tests should be conducted besides the Platelet Count Safety Panel (Appendix B), for example, if they know or suspect that the patient has a morbidity related to thrombocytopenia.

6.2.2.1. ECG

ECGs will be recorded after the patient has been resting in a supine position for at least 5 minutes. ECGs will be performed in triplicate at the times indicated in Appendix A.

6.2.2.2. DEXA Scans

Dual energy X-ray absorptiometry (DEXA) scans will be conducted at the times indicated in Appendix A, using standardized procedures and settings. For a given patient, it is preferable not to change the imaging center and machine used from that used to determine Baseline, and such a change will be considered a minor protocol deviation.

6.2.2.3. Echocardiography

Echocardiography for assessment of cardiac function will be conducted at the times indicated in Appendix A. For a given patient, it is preferable not to change the imaging center and machine used from that used to determine Baseline, and such a change will be considered a minor protocol deviation.

6.2.2.4. MRI

MRI of the liver, spleen, and bone marrow will be conducted at the times indicated in Appendix A using standardized procedures and settings. MRIs will be evaluated by independent central readers, to assess LIC, spleen iron concentration, and, if feasible, bone marrow iron concentration, as well as liver and spleen volume. For a given patient, the imaging center and machine used should not be changed from that used to determine Baseline, and such a change at Week 53 will be considered a major protocol deviation.

6.2.2.5. Ultrasound

Ultrasound of the liver and spleen will be conducted at the times indicated in Appendix A, using standardized procedures and settings, to assess liver and spleen size. For a given patient, it is preferable not to change the imaging center and machine used from that used to determine Baseline, and such a change will be considered a minor protocol deviation.

6.2.3. Disease Severity and Quality of Life Measures

6.2.3.1. Use of Chelation

Standard of care interventions, including (i) introduction of chelation – although preferably this will not occur before Day 169 (Week 25), (ii) adjustment of chelator dose or formulation – although preferably dose increases or formulation changes will not occur before Day 169 (Week 25), and (iii) temporary suspension of chelation therapy, are permitted, at the discretion of the PI. The date of each introduction of chelation or adjustment of chelator dose or formulation while a patient is on study will be documented, along with relevant details related to the chelator, including product name and dosing regimen.

6.2.3.2. Number of Transfusions

Standard of care interventions, including transfusion, are permitted, at the discretion of the Investigator. The date of each transfusion performed while a patient is on study will be documented.

6.2.3.3. NTDT-PRO©, SF-36, PGIC, ISGA, and FACIT-Fatigue

Standard application of these surveys (see Appendix E) at the times indicated in Appendix A. Preferably, these surveys should be completed before ISIS 702843 administration. If surveys are completed after the ISIS 702843 administration at that clinic visit, this will not be considered a protocol deviation that will impact efficacy.

6.2.4. Functional Status of the Patient Measure

6.2.4.1. 6MWT with Pulse Oximetry

Standard application of the 6MWT (see Appendix E) at the times indicated in Appendix A. Throughout the 6MWT, the patient will be wearing a pulse oximeter to collect oxygen saturation and heart rate data continuously throughout the test. Preferably, the 6MWT with pulse oximetry should be completed before ISIS 702843 administration. If the 6MWT with pulse oximetry is completed after the ISIS 702843 administration at that clinic visit, this will not be considered a protocol deviation that will impact efficacy.

6.3. Restriction on the Lifestyle of Patients

6.3.1. Contraception Requirements

Male patients must refrain from sperm donation and either be abstinent† or, if engaged in sexual relations with a woman of child-bearing potential (WOCBP), the patient must use a highly effective contraception method from the time of signing the informed consent form until at least 13 weeks after their last dose of ISIS 702843. Highly effective contraception for male patients comprises a vasectomy with negative semen analysis at follow-up, or the female partner is using a highly effective contraception method. Highly-effective contraception for WOCBP partners of male patients is described in the next paragraph. Male patients with partners who are pregnant must use condoms to ensure that the fetus is not exposed to ISIS 702843.

WOCBP patients must refrain from egg donation and either be abstinent† or use a highly effective contraception method from the time of signing the informed consent form until at least 13 weeks after their last dose of ISIS 702843. Highly effective contraception for WOCBP patients – and WOCBP partners of male patients – comprises surgical sterilization (e.g., bilateral tubal occlusion), hormonal contraception associated with inhibition of ovulation (combined estrogen and progestogen containing, or progestogen-only contraceptives), intrauterine contraception device, or intrauterine hormone-releasing system (IUS).

†**Note:** Abstinence (i.e., refraining from heterosexual intercourse throughout the duration of study participation) is only acceptable as true abstinence, i.e., when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a trial, and withdrawal are not acceptable methods of contraception.

For the purposes of this study, WOCBP are defined as any female who has experienced menarche, and who does not meet one of the following conditions:

- Postmenopausal: 12 months of spontaneous amenorrhea in females > 55 years of age or, in females ≤ 55 years of age, 12 months of spontaneous amenorrhea without an

alternative medical cause and FSH levels in the postmenopausal range for the laboratory involved

- 6 weeks after surgical bilateral oophorectomy with or without hysterectomy
- Post hysterectomy

6.3.2. Other Requirements

All patients will be required to fast for approximately 8–10 hours before visits requiring fasted blood sampling. Due to the length of time between visits, a reminder should be communicated to the patient prior to each visit where a fasting blood sample is collected.

7. STUDY DRUG

[redacted]

7.2. Packaging and Labeling

The Sponsor will provide the Investigator with packaged ISIS 702843, labeled in accordance with specific country regulatory requirements.

7.3. Study Drug Accountability

The study staff is required to document the receipt, dispensing, and return/destruction of ISIS 702843 supplies provided by the Sponsor, according to Sponsor instruction and in accordance with institutional policy.

8. TREATMENT OF PATIENTS

8.1. ISIS 702843 Administration

Unless the allowances in the circumstance of a public health emergency apply (Section 3.8), through Day 365 (Week 53) ISIS 702843 will be administered SC at the clinic, either by trained Study Center staff or by the trained patient/caregiver under the observation of Study Center staff. After that visit – earlier if Section 3.8 applies – SC administration away from the clinic (e.g., at the patient's home) is allowed, and this may be accomplished by any trained individual, including a caregiver or the patient themselves (referred to in this protocol as "self-administration").

The planned doses and corresponding volumes to be administered are shown in Table 1. However, ISIS 702843 dose may be adjusted as described in Section 8.7.

Vials are for single use only.

Each dose will be administered [redacted] SC injection(s) in the abdomen, thigh, or outer area of the upper arm, preferably not using the same anatomical area for consecutive doses (i.e., that are 28 days apart) [redacted]. In this context, different quadrants of the abdomen are considered different anatomical areas. [redacted] Refer to the Study Drug Manual provided by the Sponsor for more detailed instructions for ISIS 702843 preparation and administration.

Table 1: Planned ISIS 702843 Dosing Information

Cohort	Volume to Administer	Dose	Number of Doses	Total Dose
Cohort A	[redacted]	120 mg ISIS 702843	Up to 26 at 120 mg, and at least 1 at 30 mg (because all Cohort A patients are randomized before Amendment 2 approval)	Up to 3,150 mg
Cohort B	[redacted]	120 mg ISIS 702843	Up to 27	Up to 3,240 mg
Cohort C	[redacted] or, after the planned dose escalation, [redacted]	120 mg or, after the planned dose escalation, 160 mg ISIS 702843	Up to 3 at 120 mg, and up to 24 at 160 mg	Up to 4,200 mg

* [redacted]

8.2. Other Protocol-Required Drugs

There are no other protocol-required drugs concurrent with ISIS 702843.

8.3. Other Protocol-Required Treatment Procedures

There are no other protocol-required treatment procedures.

8.4. Treatment Precautions

No specific treatment precautions are required.

8.5. Safety Monitoring Rules

Please refer also to the 'Guidance for Investigator' section of the Investigator's Brochure.

For the purposes of safety monitoring, Baseline is defined in Section 10.4.

In addition to the standard monitoring of clinical safety parameters, the following guidelines are provided for the monitoring of selected parameters chosen based on preclinical and clinical observations.

Confirmation Guidance: At any time during the study (Treatment or Post-Treatment), the initial clinical laboratory results meeting the safety monitoring criteria presented below **must be confirmed** by performing measurements (ideally in the same laboratory that performed the initial measurement) on new specimens. All new specimen collections should take place as soon as possible – ideally within 3 to 7 days of the initial collection – which may require an unscheduled visit or blood draw. For stopping rules, if the initial laboratory result is observed during the Treatment Period, the results from the retest **must be available** prior to administering the next dose of ISIS 702843.

Redosing Guidance: Patients with initial laboratory test values that reach a pausing or stopping rule must not be re-dosed until the retest results are available. In general, patients who do not meet the stopping rules based upon retest may continue dosing. However, the Investigator and the Sponsor Medical Monitor (or appropriately qualified designee) should confer as to whether additional close monitoring of the patient is required. If any of the stopping criteria described below (refer to Section 8.6.1 to Section 8.6.6) are met, the patient will be permanently discontinued from further treatment with ISIS 702843, evaluated fully as outlined below and in consultation with the Medical Monitor or appropriately qualified designee, and will be followed up in accordance with Section 8.8 of the protocol.

8.5.1. Safety Monitoring Rules for Liver Chemistry Tests

The following rules are adapted from the draft guidance for industry, "Drug-Induced Liver Injury: Premarketing Clinical Evaluation," issued by the U.S. Department of Health and Human Services, Food and Drug Administration, July 2009.

In the event of an ALT or AST measurement that is > the greater of (i) 2 × Baseline or (ii) 4 × ULN if Baseline is > ULN at any time during the study (Treatment or Post-Treatment), the initial measurement(s) should be confirmed as described above. Additional, confirmatory measurements should also be performed if ALT or AST levels increase to > 6 × ULN.

Frequency of Repeat Measurements: Patients with confirmed ALT or AST levels > the greater of (i) 2 × Baseline or (ii) 4 × ULN if Baseline is > ULN, should have their liver chemistry tests (ALT, AST, ALP, INR, and total bilirubin) retested at least once weekly until ALT and AST levels become ≤ (i) 1.2 × ULN or (ii) 1.2 × Baseline if Baseline is > ULN, as applicable.

Further Investigation into Liver Chemistry Elevations: For patients with confirmed ALT or AST levels > the greater of (i) 2 × Baseline or (ii) 4 × ULN if Baseline is > ULN, the following evaluations should be performed:

- Obtain a more detailed history of symptoms and prior and concurrent diseases

- Obtain further history for concomitant drug use (including nonprescription medications, herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets
- Obtain a history for exposure to environmental chemical agents and travel
- Serology for viral hepatitis (hepatitis A virus immunoglobulin M [HAV IgM], HBsAg, hepatitis C virus [HCV] antibody, Cytomegalovirus [CMV] IgM, and Epstein-Barr virus [EBV] antibody panel)
- Serology for autoimmune hepatitis (e.g., antinuclear antibody [ANA])

Additional liver evaluations, including gastroenterology/hepatology consultations, hepatic computed tomography or MRI scans, may be performed at the discretion of the Investigator, in consultation with the Medical Monitor. Repetition of the above evaluations should be considered if a patient's ALT and/or AST levels reach > 6 × ULN.

Baseline is defined in Section 10.4.

8.5.2. Safety Monitoring Rules for Platelet Count Results

Platelet count will be monitored at least every 4 weeks through Day 197 (Week 29). From then through the remainder of the Treatment Period, platelets will be monitored every 8 weeks. During the PTP, platelets will be monitored as per schedule (see Appendix A Schedule of Procedures). The Investigator should review all platelet count results within 48 hours of receipt. If a patient's platelet count falls between < 100,000/mm^3 and 75,000/mm^3, then the patient's platelet counts should be monitored at least weekly. In case of platelet reduction to < 75,000/mm^3, the platelet monitoring rules defined in Temporary and Permanent Stopping rules (Section 8.6.6) should be followed.

Any unreportable platelet count result (e.g., caused by a hemolyzed, clumped, or clotted sample) must be rechecked and determined not to have met a stopping rule before dosing can continue.

Any case of a platelet count < 50,000/mm^3 should be reported to the Sponsor in an expedited fashion (see Section 8.6.6). The Medical Monitor will discuss the assessment and treatment of such patients with the PI, considering whether any additional tests should be conducted besides the Platelet Count Safety Panel (Appendix B), for example, if they know or suspect that the patient has a morbidity related to thrombocytopenia.

8.5.3. Safety Monitoring for Minor Bleeding Events

Minor bleeding events are those that do not fulfill the criteria for major bleeding (MB) or clinically relevant non-major bleeding events (which are defined in Section 8.6.6), for example excess bruising, petechiae, gingival bleeding on brushing teeth. If a minor bleeding event occurs, additional testing of coagulation parameters (aPTT, PT, INR) and platelet count should be performed.

8.6. Temporary and Permanent Stopping Rules

For the purposes of the stopping rules, Baseline is defined in Section 10.4.

If ISIS 702843 dosing is paused (i.e., a temporary stopping rule is implemented) and later resumed, the next dose should be given at the next Study Day at which dosing is scheduled. This will cause the total number of doses that this patient will receive in the study to be fewer than the maximum number of 27.

8.6.1. Temporary Stopping Rules for Hb Results

In the event of a confirmed Hb result meeting any of the following criteria, ISIS 702843 treatment will be suspended, at least temporarily:

1. Confirmed Hb value < 6.0 g/dL and a decrease from Baseline of at least 1.0 g/dL, or if in the judgment of the Investigator the patient is clinically symptomatic, or
2. Confirmed Hb value ≥ 12.5 g/dL, or
3. Confirmed Hb increase of ≥ 2.0 g/dL within a 4-week period and Hb > 10.5 g/dL

Confirmation of a Hb result should occur before the next scheduled visit, ideally within 3 to 7 days after the initial result, which may require an unscheduled visit or blood draw.

Following temporary stopping of ISIS 702843 treatment, Hb will continue to be monitored on a monthly basis or more frequently based on the discretion of the Investigator. ISIS 702843 treatment may be resumed in the following circumstances, at the discretion of the Investigator, in consultation with the Medical Monitor:

- If treatment was suspended based on low Hb (the first criterion, above), then treatment may be resumed based on a confirmed Hb value ≥ Baseline, for example after a transfusion
- If treatment was suspended based on Hb ≥ 12.5 g/dL (the second criterion, above), then treatment may be resumed based on (i) a confirmed Hb value < 12.5 g/dL and (ii) at least 2 scheduled doses have been missed
- If treatment was suspended based on Hb increase of ≥ 2.0 g/dL within a 4-week period (the third criterion, above), then treatment may be resumed based on (i) Hb not continuing to increase and (ii) at least 2 scheduled doses have been missed

In all situations, the timing of reintroduction of treatment will be based on the Investigator's judgment on a case-by-case basis and in consultation with the Medical Monitor, ensuring that the patient is not subject to undue risk, such as from relatively rapid Hb elevations.

8.6.2. Temporary Stopping Rules for Thalassemia Intermedia Related Safety Concerns/ Morbidities

Patients will be closely monitored for increasing severity and chronicity of their disease.

If the patient develops any well recognized morbidities of Thalassemia Intermedia that are associated with increasing severity and chronicity of the disease, the Investigator, in consultation with the Medical Monitor, should consider suspending ISIS 702843 treatment, at least temporarily, for example with the following morbidities:

- Development of symptomatic PHT (i.e., exertional dyspnea), or PHT on pharmacologic treatment, or tricuspid regurgitation > 3 m/s on echocardiography

ISIS 702843 treatment may resume at the discretion of the Investigator, in consultation with the Medical Monitor.

8.6.3. Permanent Stopping Rules for Thalassemia Intermedia Related Safety Concerns / Morbidities

If the patient develops any well recognized morbidities of Thalassemia Intermedia that are associated with increasing severity and chronicity of the disease, the Investigator, in consultation with the Medical Monitor, should consider stopping ISIS 702843 treatment permanently, for example with the following morbidities:

1. Development of a clinically confirmed thrombosis, or
2. Clinical evidence of splenic rupture, or
3. Development of clinically significant heart failure (NYHA class 3 or higher), or left ventricular ejection fraction that is either < 40% absolute or ≥ 20% reduction from Baseline

8.6.4. Temporary and Permanent Stopping Rules for Liver Chemistry Elevations

In the event of a laboratory result meeting any of the following criteria, **and the event is without an alternative explanation as discussed with the Medical Monitor,** dosing of the patient with ISIS 702843 will be paused. Confirmation of a laboratory result should be completed preferably within 3 to 7 days – which may require an unscheduled visit or blood draw. If the result is confirmed by a repeat measurement, then dosing of that patient with ISIS 702843 will be stopped permanently:

1. ALT or AST > 10 x ULN, or
2. ALT or AST > 6 x ULN, which persists for ≥ 2 weeks, or
3. ALT or AST > the greater of (i) 2 x Baseline or (ii) 4 × ULN if Baseline is > ULN, and total bilirubin > 2 × ULN or INR > 1.5, or
4. ALT or AST > the greater of (i) 2 × Baseline or (ii) 4 × ULN if Baseline is > ULN, and the new appearance (i.e., onset coincides with the changes in hepatic enzymes) of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or concomitant eosinophilia (> ULN)

8.6.5. Temporary and Permanent Stopping Rules for Renal Function Test Results

In the event of a laboratory result meeting either of the following criteria, dosing of the patient with ISIS 702843 will be paused. Confirmation of a laboratory result should be completed as soon as possible – ideally within 7 days, should be within 2 weeks – which may require an unscheduled visit or blood draw. If the result is confirmed by a repeat measurement, then dosing of that patient with ISIS 702843 will be stopped permanently:

1. Serum creatinine increase that is both ≥ 0.3 mg/dL (26.5 μmol/L) and ≥ 40% above Baseline creatinine values (Baseline is defined in Section 10.4)
2. 30% decline in eGFR from Baseline eGFR values

In the event of a confirmed UPCR result meeting either of the following criteria, dosing of the patient with ISIS 702843 will be paused. Confirmation of a laboratory result should be completed as soon as possible – ideally within 7 days, should be within 2 weeks – which may require an unscheduled visit or blood and urine samples:

1. Proteinuria manifesting as UPCR ≥ 0.50 mg/mg that also represents an increase from Baseline of at least 2 ×, or
2. Proteinuria manifesting as UPCR ≥ 0.70 mg/mg

Following temporary stopping of ISIS 702843 treatment because of proteinuria, UPCR will continue to be monitored on a monthly basis or more frequently based on the discretion of the PI. ISIS 702843 treatment may be resumed at the discretion of the PI, in consultation with the Medical Monitor, and will be based on factors such as the patient's historical UPCR results in conjunction with other renal parameters.

The follow-up schedule for any events meeting any of these stopping criteria will be determined by the Investigator, in consultation with the Medical Monitor. At the discretion of the Investigator, in consultation with the Medical Monitor, a decision to hold or permanently stop ISIS 702843 treatment may be made based on lesser changes in these parameters observed in isolation or in association with other renal-related abnormalities.

8.6.6. Temporary and Permanent Stopping Rules for Platelet Count Results

In the event of any platelet count < 100,000/mm^3, monitoring frequency and dosing should be adjusted as recommended in the table below.

Table 2: Actions in Patients with Platelet Count ≤ Lower Limit of Normal (LLN)

Platelet Count	Dosing Action	Monitoring
≤ LLN to 100,000/mm^3	Continue	Monitor as per protocol
< 100,000/mm^3 to 75,000/mm^3	Continue	Monitor at least weekly
< 75,000/mm^3 to 50,000/mm^3, in the absence of major bleeding or clinically relevant non-major bleeding (defined below)	Pause	Monitor at least twice per week until 3 successive values above 100,000/mm^3. Then monitor weekly until values normalize. The suitability of the patient for continued dosing will be determined by the Investigator in consultation with the Medical Monitor and will be based on factors such as the original rate of decline in the patient's platelet count, whether any bleeding events were experienced, and the speed of recovery of platelet count after the dose pause. The patient must have 3 successive values above 100,000/mm^3 before continued dosing is considered.
< 50,000/mm^3 to 25,000/mm^3	Pause	Report to the Sponsor in an expedited fashion Monitor at least twice per week until 3 successive values above 75,000/mm^3. Then monitor weekly until values normalize. A hematologist consultation may be considered at the discretion of the Investigator and Medical Monitor Administration of corticosteroids* and a hematologist consultation is recommended
< 25,000/mm^3 to 10,000/mm^3	Permanently Discontinue	Report to the Sponsor in an expedited fashion Monitor daily until 2 successive values above 25,000/mm^3. Then monitor twice per week until 3 successive values above 75,000/mm^3. Then monitor weekly until values normalize. Administration of corticosteroids* and a hematologist consultation is recommended
< 10,000/mm^3	Permanently Discontinue	Report to the Sponsor within 24 hours Monitor daily until 2 successive values above 25,000/mm^3. Then monitor twice per week until 3 successive values above 75,000/mm^3. Then monitor weekly until values normalize. Hospitalize patient and expedite a hematologist consultation Administer platelet transfusions and corticosteroids*, if deemed necessary

* Treatment guidelines for immune thrombocytopenia (Provan et al. 2010) recommend dexamethasone 40 mg daily for 4 days every 2-4 weeks for 1-4 cycles; prednis(ol)one 0.5-2 mg/kg/d for 2-4 weeks then taper; or parenteral methylprednisolone 30 mg/kg/day for 7 days. (Note: Patient may require continuation with oral corticosteroids after methylprednisolone)

In the event of any platelet count > 1,000,000/mm^3, the utility of adjustments to concomitant medications (e.g., anti-coagulants) will be determined by the Investigator in consultation with the Medical Monitor.

Definition of Major Bleeding Events (Schulman and Kearon 2005):

1. Fatal bleeding, and/or
2. Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intraarterial or pericardial, or intramuscular with compartment syndrome

3. Clinically overt bleeding leading to transfusion of ≥ 2 units of packed RBC or whole blood or a fall in Hb of 2.0 mg/dL (1.24 mmol/L) or more within 24 hours

Definition of Clinically Relevant Non-Major Bleeding Events

Clinically relevant non-major bleeding (CRNMB) is defined as overt bleeding not meeting the criteria for MB but that resulted, for example, in medical examination, intervention, or had clinical consequences for a patient.

Definition of Minor Bleeding Events

Minor bleeding events are those that do not fulfill the criteria for MB or CRNMB events (defined above), for example excess bruising, petechiae, gingival bleeding on brushing teeth.

8.7. Adjustment of ISIS 702843 Dose and/or Treatment Schedule

Dose pauses are permitted, as described in Section 8.6.

Two (2) interim analyses are planned. The first interim analysis is planned to occur when at least 20 patients have completed evaluations associated with Day 183 (Week 27). The second interim analysis is planned to occur when at least 16 patients have completed evaluations associated with Day 365 (Week 53). Based on these and/or other analysis(es), ISIS 702843 dose adjustments in addition to the dose escalation to 160 mg planned in Cohort C (Section 3.1) will be permitted using the criteria numbered 1–4 below as a guide if the data support such a change providing additional therapeutic benefit or safety to the patient(s) in the judgment of the Investigator, in consultation with the Medical Monitor. These adjustments may be applicable to:

- An entire cohort, but only after the first interim analysis
- An individual patient, but only after that individual has completed PK and PD evaluations associated with Day 281 (Week 41)

Earlier adjustment(s) for an individual patient may occur if it is deemed necessary, and the Investigator should discuss these proposed adjustment(s) with the Medical Monitor prior to implementation.

Standard of care interventions, including transfusion, introduction of iron chelation therapy, and adjustment of chelator dose or formulation, will be permitted, at the discretion of the Investigator. For example, if serum ferritin decreases to the extent that over-chelation is a concern, then it is expected that the Investigator will consider reducing the chelator dose, as appropriate, before consideration is given to adjusting the ISIS 702843 dose.

ISIS 702843 dose adjustments should be considered by the Investigator, in consultation with the Medical Monitor, using the following criteria numbered 1-4 below as a guide. Allowable dose levels for all cohorts are 30, 50, and 80 mg (i.e., the initial dose levels for Cohorts A, B, and C, respectively, before Amendment 2), and 120 mg (i.e., the initial dose level planned for Cohorts A, B, and C after Amendment 2 approval), and 160 mg [i.e., the dose level planned for Cohort C after dose escalation, if allowed based on a demonstration of adequate safety (Section 3.1), after Amendment 2 approval]. Multiple adjustments (increases and decreases) per subject are allowed if the data support such changes providing additional therapeutic benefit or safety to the patient(s) in the judgment of the PI, in consultation with the Medical Monitor.

1. If ISIS 702843 treatment was suspended based on decreased Hb (the first criterion in Section 8.6.1), follow standard of care measures including considering transfusing the patient; then, if ISIS 702843 treatment resumes and it is after Day 281 (Week 41) for this patient, consider other Hematology and Iron Metabolism Panel measures to determine whether the ISIS 702843 dose should be adjusted from the prior dose level. When increasing or decreasing the ISIS 702843 dose for an individual patient, the change should not exceed one dose level from the previous, i.e., make one of the following changes: from 30 mg to 50 mg, from 50 mg to 80 mg, from 80 mg to 120 mg, from 120 mg to 160 mg, from 160 mg to 120 mg, from 120 mg to 80 mg, from 80 mg to 50 mg, or from 50 mg to 30 mg. (Exceptions to the practice of not exceeding one dose level change per dose adjustment are the following, which are based on Amendment 2: the Cohort A dose increase from 30 mg to 120 mg, and the Cohort B dose increase from 50 mg to 120 mg.)
2. If ISIS 702843 treatment was suspended based on increased Hb (the second or third criteria in Section 8.6.1), then, if ISIS 702843 treatment resumes and it is after Day 281 (Week 41) for an individual patient, consider decreasing the ISIS 702843 dose to the next dose level, as applicable, i.e., from 160 mg to 120 mg, from 120 mg to 80 mg, from 80 mg to 50 mg, or from 50 mg to 30 mg
3. If a patient is not exhibiting a notable PD effect (e.g., increase in Hb or decrease in LIC) through Day 281 (Week 41) or later, then consider increasing the ISIS 702843 dose to the next dose level, as applicable, i.e., from 30 mg to 50 mg, from 50 mg to 80 mg, from 80 mg to 120 mg, or from 120 mg to 160 mg.
4. If, at Day 281 (Week 41), Day 365 (Week 53), or Day 533 (Week 77), LIC ≤ 3.0 mg Fe/g dry weight, and serum ferritin ≤ 300 ng/mL, follow standard of care measures including first consider reducing or suspending chelator treatment, at least temporarily, and only thereafter consider decreasing the ISIS 702843 dose to the next dose level, as applicable, i.e., from 160 mg to 120 mg, from 120 mg to 80 mg, from 80 mg to 50 mg, or from 50 mg to 30 mg

Maximum ISIS 702843 dose will not exceed 160 mg per administration, with a planned 28-day interval between each dose (Section 2.4).

8.8. Discontinuation of ISIS 702843 Treatment

A patient must permanently discontinue ISIS 702843 treatment for any of the following:

- The patient becomes pregnant. Report the pregnancy according to instructions in Section 9.5.4
- The patient withdraws consent
- The patient experiences an AE that in the opinion of the Investigator, in consultation with the Medical Monitor, necessitates permanent discontinuation of ISIS 702843 treatment
- The patient develops laboratory test abnormalities that meet any of the permanent stopping rules listed in Section 8.6.1 to Section 8.6.6

The reason for discontinuation of ISIS 702843 treatment must be recorded in the electronic Case Report Form (eCRF) and source documentation.

Patients who discontinue from ISIS 702843 treatment and agree to remain in the study will attend the following clinic visits: ETFT, PTP Visit 1, PTP Visit 2, and PTP Last Visit. If the patient is unable or unwilling to participate in all of these visits, the Investigator should determine the follow-up procedures the patient would agree to complete. Every effort should be made to complete the ETFT procedures and observations at the time of withdrawal.

8.9. Withdrawal of Patients from the Study Procedures

Patients must be withdrawn from study procedures for any of the following:

- Withdrawal of consent
- The patient is unwilling or unable to comply with the protocol

Other reasons for withdrawal of patients from study procedures might include:

- At the discretion of the Investigator for medical reasons
- At the discretion of the Investigator or Sponsor for noncompliance
- Significant protocol deviation

All efforts will be made to complete and report the observations as thoroughly as possible up to the date of withdrawal. All information, including the reason for withdrawal from study, must be recorded in the eCRF.

Any patient who withdraws consent to participate in the study will be removed from further treatment and study observation immediately upon the date of request. These patients should be encouraged to complete the early termination study procedures and observations at the time of withdrawal (Appendix A).

For patients withdrawn for reasons other than withdrawal of consent, every effort should be made to complete the early termination of study procedures and observations at the time of withdrawal (see Appendix A) and ideally within 4 weeks from the last dose of ISIS 702843.
If the patient declines or is unable to participate in the above, the Investigator should determine the follow-up procedures the patient would agree to complete. Wherever possible, these patients should continue to be followed up via the agreed means to collect information on AEs, concomitant medications, and survival status. At the very least, the patient's status at the end of the protocol defined Study Period should be ascertained and documented wherever possible. The agreed means of follow-up will be documented in the patient records and notified to the Sponsor.

8.10. Concomitant Therapy and Procedures

The use of concomitant therapies or procedures defined below must be recorded on the patient's eCRF. Adverse events related to administration of these therapies or procedures must also be documented on the appropriate eCRF.

8.10.1. Concomitant Therapy

A concomitant therapy is any non-protocol specified drug or substance (including over-the-counter medications, herbal medications, vitamin supplements, and iron supplements) administered at any time from Screening through the end of the PTP.

Allowed Concomitant Therapies

Standard-of-care interventions are permitted at the discretion of the PI, and these may include:

- Blood transfusions
- Introduction of iron chelation therapy – although preferably this will not occur before Day 169 (Week 25)
- Adjustment of chelator dose or formulation – although preferably dose increases or formulation changes will not occur before Day 169 (Week 25)
  - For example, if serum ferritin decreases to the extent that over-chelation is a concern, then it is expected that the Investigator will consider reducing the chelator dose, as appropriate

Also allowed is treatment with either luspatercept-aamt (Reblozyl®) or EPO, not both, after Day 365 (Week 53).

Disallowed Concomitant Therapy

Use of iron supplements, including iron-containing vitamins, is not allowed.

8.10.2. Concomitant Procedures

A concomitant procedure is any therapeutic intervention (e.g., surgery/biopsy, physical therapy) or diagnostic assessment (e.g., blood gas measurement, bacterial cultures) performed at any time from Screening through the end of the PTP.

Allowed Concomitant Procedures

Standard-of-care procedures are permitted at the discretion of the Investigator.

Disallowed Concomitant Procedure

There are no disallowed concomitant procedures.

8.11. Treatment Compliance

Compliance with treatment dosing is to be monitored and recorded in the eCRF by Study Center staff.

Self-administration will be documented by the patients or (applicable if a caregiver administered the dose) caregivers in a dosing diary that will be reviewed periodically by Study Center staff and the Clinical Monitor.

9. SERIOUS AND NON-SERIOUS ADVERSE EVENT REPORTING

9.1. Sponsor Review of Safety Information

Safety information will be collected, reviewed, and evaluated by the Sponsor or designee in accordance with the applicable Ionis and/or designee SOPs throughout the conduct of the clinical trial.

Listings of serious adverse events (SAEs) will be provided to the internal safety committee; potential safety issues will be reviewed based on the internal safety committee Charter.

9.2. Regulatory Requirements

The Sponsor or designee is responsible for regulatory submissions and reporting to the Investigators of SAEs including suspected unexpected serious adverse reactions (SUSARs) per the International Conference on Harmonization (ICH) guidelines E2A and ICH GCP. Country-specific regulatory requirements will be followed in accordance with local country regulations and guidelines.

Ethics Committees / Institutional Review Boards will be notified of any SAE according to applicable regulations.

In addition to the Investigator's assessment of relatedness, the Sponsor or designee will evaluate the available information and perform an independent assessment of all reported SAEs and determine if there is a reasonable possibility that ISIS 702843 is causally related to a reported SAE. While the Sponsor may upgrade an Investigator's decision, it is not permissible to downgrade the Investigator's opinion for the purposes of determining whether the SAE meets the definition of a SUSAR.

9.3. Definitions

9.3.1. Adverse Event

An adverse event (AE) can be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of medicinal (investigational) product, whether or not the AE is considered related to the medicinal (investigational) product.

An AE can therefore be any of the following:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product
- Any new disease or exacerbation of an existing disease (a worsening in the character, frequency, or severity of a known condition)
- Recurrence of an intermittent medical condition (e.g., headache) not present at Baseline

- Any deterioration in a laboratory value or other clinical test (e.g., ECG, X-ray) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from Study Drug
- Adverse events that are related to a protocol-mandated intervention, including those that occur prior to assignment of study treatment (e.g., screening invasive procedures such as biopsies)

9.3.2. Adverse Drug Reaction and Suspected Unexpected Adverse Drug Reaction

Adverse Drug Reaction (ADR)

In the *pre-approval* clinical experience with a new medicinal product or its new usages, particularly as the therapeutic dose(s) may not have been established, ADR is defined as follows:

> All noxious and unintended responses to a medicinal product related to any dose should be considered adverse drug reactions.

The phrase "responses to a medicinal product" means that a causal relationship between the medicinal product and the AE has been determined by the Sponsor as at least a reasonable possibility, i.e., the relationship cannot be ruled out.

Suspected Unexpected Adverse Drug Reaction

A suspected unexpected ADR is any ADR, the nature or severity of which is not consistent with the applicable product information, e.g., Investigator's Brochure, for an unapproved medicinal (investigational) product.

A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

9.3.3. Serious Adverse Event (SAE)

A SAE is any AE that in the view of either the Investigator or Sponsor, meets any of the following criteria:

- Results in death
- Is life threatening: that is, poses an immediate risk of death at the time of the event
  - An AE or suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator or Sponsor, its occurrence places the patient at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death
- Requires inpatient hospitalization or prolongation of existing hospitalization
  - Hospitalization is defined as an admission of greater than 24 hours to a medical facility and does not always qualify as an AE
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions

- Results in a congenital anomaly or birth defect in the offspring of the patient (whether the patient is male or female)
- Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse

The terms "severe" and "serious" are not synonymous. Severity refers to the *intensity* of an AE (e.g., rated as mild, moderate, or severe, or according to National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAE]), while seriousness refers to the *medical significance*. For example, a headache without any further findings has relatively minor medical significance, but it may be a severe headache.

Severity and seriousness need to be independently assessed for each AE recorded on the eCRF.

9.4. Monitoring and Recording Adverse Events

Any pre-existing conditions or signs and/or symptoms present in a patient prior to the start of the study (i.e., before informed consent) should be recorded as Medical History and not recorded as AEs unless the pre-existing condition worsened. The Investigator should always group signs and symptoms into a single term that constitutes a **single unifying diagnosis** if possible. Before a diagnosis is confirmed, all symptoms should be reported as separate AEs.

9.4.1. Serious Adverse Events

In the interest of patient safety, and in order to fulfill regulatory requirements**,** all SAEs (regardless of their relationship to ISIS 702843) should be reported to the Sponsor or designee within 24 hours of the Study Center's first knowledge of the event. The collection of SAEs will begin after the patient signs the informed consent form and stop at the end of the patient's Follow-up Period which is defined as PTP Last Visit. When the Investigator is reporting by telephone, it is important to speak to someone in person versus leaving a message. Once the patient is randomized, SAEs should be reported using an electronic SAE submission form whenever possible. In situations where the electronic SAE submission is unavailable or an SAE occurs prior to the patient's randomization, a paper Initial Serious Adverse Event Form should be completed and a copy should be faxed or emailed to the Sponsor or designee. For SAEs reported after randomization, information submitted on the paper form should be entered into EDC as soon as the system becomes available. The SAE reporting instructions, including the fax number and email address can be found in the Investigator site file for the study.

Detailed follow-up information should be actively sought and promptly submitted electronically, or on a Serious Adverse Event Form if reporting by paper, before randomization. All SAEs will be followed until resolution. SAEs that remain ongoing past the patient's last protocol-specified follow-up visit will be evaluated by the Investigator and Sponsor. If the Investigator and

Sponsor agree the patient's condition is unlikely to resolve, the Investigator and Sponsor will determine the follow-up requirement.

9.4.2. Non-Serious Adverse Events

The recording of non-serious AEs will begin after the patient signs the informed consent form and will stop at the end of the patient's Follow-up Period, which is defined as PTP Last Visit. The Investigator will monitor each patient closely and record all observed or volunteered AEs on the Adverse Event Case Report Form.

9.4.3. Evaluation of Adverse Events (Serious and Non-Serious)

The Investigator's opinion of the following should be documented on the Adverse Event Case Report Form:

9.4.3.1. Relationship to ISIS 702843

The event's relationship to ISIS 702843 is characterized by one of the following:

- **Related:** There is clear evidence that the event is related to the use of ISIS 702843, e.g., confirmation by positive re-challenge test
- **Possible:** The event cannot be explained by the patient's medical condition, concomitant therapy, or other causes, and there is a plausible temporal relationship between the event and ISIS 702843 administration
- **Unlikely/Remote:** An event for which an alternative explanation is more likely (e.g., concomitant medications or ongoing medical conditions) or the temporal relationship to ISIS 702843 administration and/or exposure suggests that a causal relationship is unlikely (For reporting purposes, Unlikely/Remote will be grouped together with Not Related)
- **Not Related:** The event can be readily explained by the patient's underlying medical condition, concomitant therapy, or other causes, and therefore, the Investigator believes no relationship exists between the event and ISIS 702843

9.4.3.2. Severity

The severity of AEs and SAEs relating to laboratory tests and adverse events at the injection site may be graded based on criteria from the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, November 2017 (refer to Appendix D).

Alternatively, the Investigator should classify events and laboratory findings as mild, moderate, or severe based on the clinical significance of the event and laboratory finding for that patient, for example, laboratory findings related to Hb, ALT, and AST.

Any AE not listed in Appendix D will be graded as follows:

- **Mild:** The event is easily tolerated by the patient and does not affect the patient's usual daily activities

- **Moderate:** The event causes the patient more discomfort and interrupts the patient's usual daily activities
- **Severe:** The event is incapacitating and causes considerable interference with the patient's usual daily activities

If the event is an SAE, then all applicable seriousness criteria must be indicated (criteria listed in Section 9.3.3).

9.4.3.3. Action Taken with ISIS 702843

Action taken with ISIS 702843 due to the event is characterized by one of the following:

- **None:** No changes were made to ISIS 702843 administration and dose
- **Not Applicable:** SAE/AE was reported during Screening Period prior to ISIS 702843 administration
- **Permanently Discontinued:** ISIS 702843 was discontinued and not restarted
- **Temporarily Interrupted, Restarted – Same Dose:** Dosing and/or dosing frequency was temporarily interrupted/changed or delayed due to the AE and restarted at the same dose
- **Reduced Dose:** Dosing was reduced, temporarily interrupted, or delayed due to the AE and restarted at the next lower dose or reduced dosing frequency

9.4.3.4. Treatment Given for Adverse Event

Any treatment (e.g., medications or procedures) given for the AE should be recorded on the Adverse Event Case Report Form. Treatment should also be recorded on the concomitant treatment or ancillary procedures eCRF, as appropriate.

9.4.3.5. Outcome of the Adverse Event

If the event is a non-serious AE, then the event's outcome is characterized by one of the following:

- **AE Persists:** Patient terminates from the trial and the AE continues
- **Recovered:** Patient recovered completely from the AE
- **Became Serious:** The event became serious (the date that the event became serious should be recorded as the Resolution Date of that AE and the Onset Date of the corresponding SAE)
- **Change in Severity** (if applicable): AE severity changed

If the event is an SAE, then the event's outcome is characterized by one of the following:

- **Ongoing:** SAE continuing
- **Persists (as non-serious AE):** Patient has not fully recovered but the event no longer meets serious criteria and should be captured as an AE on the non-serious AE eCRF (the SAE resolution date should be entered as the date of onset of that AE)

- **Recovered:** Patient recovered completely from the SAE (the date of recovery should be entered as the SAE resolution date)
- **Recovered with Sequelae:** The signs/symptoms of the reported SAE have improved but not completely resolved, and a new baseline for the patient is established since full recovery is not expected
- **Fatal:** Patient died (the date of death should be entered as the SAE resolution date)
- **Unknown:** The outcome of the reported SAE is not available, e.g., patient is lost to follow-up

9.4.3.6. Follow-up of Adverse Event

Investigator Follow-Up

During the Study Period, the Investigator should follow each AE until the event has resolved to baseline grade or better, the event is assessed as stable, the patient is lost to follow-up, or the patient withdraws consent. Every effort should be made to follow all SAEs considered to be related to ISIS 702843 or related to study procedures until a final outcome can be reported.

Resolution of AE (with dates) should be documented on the Adverse Event eCRF and in the patient's medical record to facilitate source data verification.

Investigator should follow-up, or support the Sponsor's effort to follow up with all pregnancies reported during the study from either the study patient or the female partner of male study patient until pregnancy outcome is available.

Sponsor Follow-Up

For SAEs and pregnancy cases in patients who have completed or terminated study, the Sponsor or a designee should follow-up by telephone, fax, email, and/or a monitoring visit to obtain additional case details and outcome information (e.g., from hospital discharge summaries, consultant reports, autopsy reports) in order to perform an independent medical assessment of the reported case.

9.5. Procedures for Handling Special Situations

9.5.1. Abnormalities of Laboratory Tests

Clinically significant abnormal laboratory test results may, in the opinion of the Investigator, constitute or be associated with an AE. Examples of these include abnormal laboratory results that are associated with symptoms, or require treatment, e.g., bleeding due to thrombocytopenia, tetany due to hypocalcemia, or cardiac arrhythmias due to hyperkalemia. Whenever possible, the underlying diagnosis should be listed in preference to abnormal laboratory values as AEs. Clinically significant abnormalities will be monitored by the Investigator until the parameter returns to its baseline value or until agreement is reached between the Investigator and Medical Monitor. Laboratory abnormalities deemed not clinically significant (NCS) by the Investigator should not be reported as AEs. Similarly, laboratory abnormalities reported as AEs by the Investigator should be deemed CS on the laboratory sheet.

The Investigator is responsible for reviewing and signing all laboratory reports. The signed clinical laboratory reports will serve as source documents and should include the Investigator's assessment of clinical significance of out of range/abnormal laboratory values.

9.5.2. Prescheduled or Elective Procedures or Routinely Scheduled Treatments

A prescheduled or elective procedure or a routinely scheduled treatment will not be considered an SAE, even if the patient is hospitalized; the Study Center must document all of the following:

- The prescheduled or elective procedure or routinely scheduled treatment was scheduled (or was on a waiting list to be scheduled) prior to obtaining the patient's consent to participate in the study
- The condition that required the prescheduled or elective procedure or routinely scheduled treatment was present before and did not worsen or progress in the opinion of the Investigator between the patient's consent to participate in the study and the timing of the procedure or treatment
- The prescheduled or elective procedure or routinely scheduled treatment is the sole reason for the intervention or hospital admission

9.5.3. Dosing Errors

ISIS 702843 dosing errors (including overdose, underdose, and administration error) should be documented as Protocol Deviations. A brief description should be provided in the deviation, including whether the patient was symptomatic (list symptoms) or asymptomatic, and the event accidental or intentional.

Dosing details should be captured on the Dosing Case Report Form. If the patient takes a dose of ISIS 702843 that exceeds protocol specifications and the patient is symptomatic, then the symptom(s) should be documented as an AE and be reported per Section 9.4.

An overdose is the accidental or intentional use of a drug in an amount higher than the dose being studied. An overdose or incorrect administration of study treatment is not itself an AE, but it may result in an AE. All AEs associated with an overdose or incorrect administration of ISIS 702843 should be recorded on the Adverse Event eCRF. If the associated AE fulfills seriousness criteria, the event should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event).

Should an overdose occur, the Investigator or designee should refer to the Guidance to Investigator's section of the Investigator's Brochure and contact the Sponsor or designee within 24 hours.

9.5.4. Contraception and Pregnancy

Male patients and female patients of childbearing potential must continue to use highly effective contraception with their partners, or refrain from sexual activity, as described in Section 6.3.1.

If a patient becomes pregnant or a pregnancy is suspected, or if a male patient makes or believes that he has made someone pregnant during the study, then the Study Center staff must be informed immediately. An Initial Pregnancy Form should be submitted to the Sponsor or

designee **within 24 hours** of first learning of the occurrence of pregnancy. Follow-up information including delivery or termination is reported by designating as 'Follow-up' on the Pregnancy Forms and reported within 24 hours.

Payment for all aspects of obstetrical care, child or related care will be the patient's responsibility.

Female patients: If a suspected pregnancy occurs while on the study (including follow-up), a pregnancy test will be performed. The patient with a confirmed pregnancy will be immediately withdrawn from treatment with ISIS 702843. However, the patient will be encouraged to complete the post-treatment follow-up portion of the study to the extent that study procedures do not interfere with the pregnancy. Regardless of continued study participation, the progress of the pregnancy will be followed until the outcome of the pregnancy is known (i.e., delivery, elective termination, or spontaneous abortion). If the pregnancy results in the birth of a child, the Study Center and Sponsor may require access to the mother and infant's medical records to obtain additional information relevant to the pregnancy progress and outcome. A longer follow-up may be required if a newborn child experiences a medical condition. Follow-up will be performed to the extent permitted by the applicable regulations and privacy considerations; e.g., pregnancy informed consent form may be required.

Male patients: The progress of the pregnancy of a male patient's partner should be followed until the outcome of the pregnancy is known (i.e., delivery, elective termination, or spontaneous abortion). If the pregnancy results in the birth of a child, **the Study Center and Sponsor may follow-up with the mother and may request access to the mother and infant's medical records** to obtain additional information relevant to the pregnancy progress and outcome. A longer follow-up may be required if a newborn child experiences a medical condition. Follow-up will be performed to the extent permitted by the applicable regulations and privacy considerations; e.g., partner pregnancy informed consent form may be required.

10. STATISTICAL CONSIDERATIONS

10.1. Stratification

Eligible patients will be stratified based on Screening Mean Hb for Eligibility (defined in Section 6.1.1), low (≤ 8.0 g/dL) and high (> 8.0 g/dL).

10.2. Sample Size Considerations

There is no statistical rationale for the selected sample size of the study dose treatment cohorts. The sample size was based on prior experience to ensure that the safety, tolerability, PK, and PD of ISIS 702843 will be adequately assessed while minimizing unnecessary patient exposure. The study will enroll approximately 24 evaluable patients overall, with approximately 12 evaluable patients in Cohorts A and B combined, and approximately 12 evaluable patients in Cohort C, following a prespecified randomization schema.

10.3. Populations

Safety Set: All randomized patients who received at least 1 dose of ISIS 702843.

PK Set: All randomized patients who received at least 1 dose of ISIS 702843 and have at least 1 evaluable PK sample.

Full Analysis Set (FAS): All randomized patients who received at least 1 dose of ISIS 702843 and who have at least 1 Hb assessment collected after Day 1.

Other populations will be defined in the SAP.

10.4. Definitions of Baseline

For all attributes other than Hematology parameters, Baseline is defined as the last measurement prior to first dose of ISIS 702843.

For hematology parameters, Baseline is defined as the mean of all measurements taken in the Screening Period and Day 1, pre-dose. Each measurement used for the calculation of Baseline must be taken at least 6 weeks after the most recent transfusion for that patient. Baseline must be based on at least 2 measurements.

10.5. Interim Analyses

Two (2) interim analyses are planned:

- The first interim analysis is planned to occur when at least 20 patients have completed evaluations associated with Day 183 (Week 27). The main purpose of this analysis is to assess Hb, other Hematology measures, and Iron Metabolism Panel measures, with this information being used to support decisions related to the potential for dose adjustments applicable to an entire cohort (see Section 8.7).
- The second interim analysis is planned to occur when at least 16 patients have completed evaluations associated with Day 365 (Week 53). The main purposes of this analysis are to assess LIC, and to further assess the measures associated with the first interim analysis.

An internal safety committee (Section 3.6) will independently review safety, tolerability, and, as needed, efficacy data collected during this study. Based on its ongoing assessment of safety, tolerability, and efficacy of ISIS 702843, this committee will provide recommendations to the study team for modifying, stopping, or continuing the study as planned.

10.6. Planned Methods of Analysis

All eCRF data, laboratory data transfers, and any outcomes derived from the data will be provided in the patient data listings. Patient data listings will be presented for all patients randomized into the study.

Descriptive summary statistics including n, mean, median, standard deviation, interquartile range (25^{th} percentile, 75^{th} percentile), and range (minimum, maximum) for continuous variables, and counts and percentages for categorical variables will be used to summarize most data by cohort and total.

10.6.1. Demographic and Baseline Characteristics

Demographic and Baseline characteristics will be summarized using descriptive statistics by cohort and total. Patient randomization and disposition will be summarized by cohort and total. All patients randomized will be included in a summary of patient disposition.

10.6.2. Safety Analysis

Treatment duration and amount of ISIS 702843 received will be summarized by cohort and total, as well as reasons for withdrawal from ISIS 702843 treatment.

Patient incidence rates of all treatment-emergent adverse events (AEs with onset after the first dose of ISIS 702843) and SAEs will be tabulated by cohort and total using Medical Dictionary for Regulatory Activities (MedDRA™) coding system by system organ class, preferred term, relationship to ISIS 702843, and severity. Narratives of deaths, serious and significant TEAEs, including early study withdrawals due to AEs, will also be provided.

Laboratory tests to ensure patient safety, including chemistry panel, complete blood count (CBC) with differential, and coagulation panel, will be summarized by study visits for each cohort. These safety variables will also be presented as change and percent change from Baseline over time after ISIS 702843 administration, as appropriate. In addition, the number of patients who experience abnormalities in clinical laboratory evaluations will be summarized.

Vital sign and ECG measures will be tabulated by cohort and total.

10.6.3. Efficacy Analyses

The primary efficacy analysis for proportion of patients achieving ≥ 1.0 g/dL increase from Baseline in Hb at Week 27 will be summarized descriptively by cohort. Additional summary by the stratification factor will also be provided.

Secondary efficacy analyses will be performed in a similar way to primary efficacy analysis, and will include:

- Proportion of patients achieving ≥ 1.5 g/dL increase from Baseline in Hb at Week 53
- Proportion of patients achieving ≥ 1.0 mg Fe/g dry weight decrease from Baseline in LIC at Week 53

Exploratory efficacy analyses will be performed in a similar way to primary efficacy analysis, and will include:

- Mean change (absolute and percentage) from Baseline with respect to each scheduled visit after Day 1, for the parameters in each of the following categories (see Section 1.2.3):
  - IE
  - Measures of iron overload
  - Hepcidin
  - Measures of anemia

Details will be presented in the SAP.

10.6.4. Pharmacokinetic Analysis

The plasma PK of ISIS 702843 (as total full-length ASO, including fully conjugated, partially conjugated, and unconjugated ISIS 702843) will be assessed following SC administration. Non-compartmental PK analysis of ISIS 702843 (as total full-length ASO) will be carried out on each individual patient data set.

Other plasma PK parameters, as appropriate, may be determined or calculated at the discretion of the PK scientist.

Metabolite identification and profiling may be conducted on select plasma samples.

Plasma PK parameters will be summarized using descriptive statistics. Additional details regarding the PK analysis will be described in the SAP.

Analysis of potential exposure-response relationship between ISIS 702843 and relevant biomarkers may be explored, as deemed appropriate.

10.6.5. Additional Exploratory Analyses

Analysis of the parameters in the categories (see Section 1.2.3) listed below will be conducted in a similar manner as described in Section 10.6.3 for mean change (absolute and percentage) from Baseline with respect to each scheduled visit after Day 1. Details will be presented in the SAP.

- Hemolysis
- Coagulation profile changes
- Disease severity and quality of life measures
- Functional status of the patient

11. INVESTIGATOR'S REGULATORY OBLIGATIONS

11.1. Informed Consent

The written informed consent document should be prepared in the language(s) of the potential patient population, based on an English version provided by the Sponsor or designee.

Before a patient's participation in the trial, the Investigator is responsible for obtaining written informed consent from the patient after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study, and before any protocol-specific screening procedures or any ISIS 702843 is administered. The patient must be given sufficient time to consider whether to participate in the study.

The acquisition of informed consent and the patient's agreement or refusal to notify his/her primary care physician should be documented in the patient's medical records, and the informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion (not necessarily an Investigator). The original signed informed consent form should be retained in the Study Master File and in any other locations

required by institutional policy, and a copy of the signed consent form should be provided to the patient.

11.2. Ethical Conduct of the Study

All applicable regulations and guidelines of current Good Clinical Practice (GCP) as well as the demands of national drug and data protection laws and other applicable regulatory requirements must be followed.

11.3. Independent Ethics Committee / Institutional Review Board

A copy of the protocol, proposed informed consent form, other written patient information, and any proposed advertising material must be submitted to the EC / Institutional Review Board (IRB) for written approval. A copy of the written approval of the protocol and informed consent form must be received by the Sponsor before recruitment of patients into the study and shipment of ISIS 702843. A copy of the written approval of any other items/materials that must be approved by the Study Center or EC / IRB must also be received by the Sponsor before recruitment of patients into the study and shipment of ISIS 702843. The Investigator's Brochure must be submitted to the EC / IRB for acknowledgement.

The Investigator must submit to and, where necessary, obtain approval from the EC / IRB for all subsequent protocol amendments and changes to the informed consent document. The Investigator should notify the EC / IRB of deviations from the protocol in accordance with ICH GCP. The Investigator should also notify the EC / IRB of SAEs occurring at the Study Center and other AE reports received from the Sponsor, in accordance with local procedures.

The Investigator will be responsible for obtaining annual EC / IRB approval/renewal throughout the duration of the study. Copies of the Investigator's reports, all EC / IRB submissions and the EC / IRB continuance of approval must be sent to the Sponsor.

11.4. Patient Confidentiality

The Investigator must ensure that the patient's confidentiality is maintained. On the CRFs or other documents submitted to the Sponsor or designee, patients should be identified by initials (if permitted by local law) and a patient identification number only. Documents that are not for submission to the Sponsor or designee (e.g., signed informed consent forms) should be kept in strict confidence by the Investigator.

The Investigator must comply with all applicable National and local regulations / ICH GCP Guidelines, including US Food & Drug regulations and, where applicable, the European Union's General Data Protection Regulation. In compliance with such regulations, it is required that the Investigator and institution permit authorized representatives of the company, of the regulatory agency(s), and the EC / IRB direct access to review the patient's original medical records for verification of study-related procedures and data. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study. The Investigator is obligated to inform and obtain the consent of the patient to permit named representatives to have access to his/her study-related records without violating the confidentiality of the patient.

12. ADMINISTRATIVE AND LEGAL OBLIGATIONS

12.1. Protocol Amendments

Protocol amendments must be made only with the prior approval of the Sponsor. Agreement from the Investigator must be obtained for all protocol amendments and amendments to the informed consent document. The regulatory authority and EC / IRB must be informed of all amendments and give approval for any amendments likely to affect the safety of the patients or the conduct of the trial. The Investigator **must** send a copy of the approval letter from the EC / IRB to the Sponsor.

12.2. Study Termination

The Sponsor reserves the right to terminate the study. The Investigator reserves the right to terminate their participation in the study, according to the terms of the site contract. The Investigator / Sponsor should notify the EC / IRB in writing of the trial's completion or early termination, and send a copy of the notification to the Sponsor.

12.3. Study Documentation and Storage

An eCRF utilizing an Electronic Data Capture (EDC) application will be used for this study.

The Investigator should ensure that all appropriately qualified persons to whom he/she has delegated trial duties are recorded on a Sponsor-approved Delegation of Site Responsibilities Form.

Source documents are original documents, data, and records from which the patient's CRF data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, imaging, and correspondence. In this study, eCRFs may not be used as source documents.

The Investigator and Study Center staff are responsible for maintaining a comprehensive and centralized filing system of all study-related (essential) documentation in accordance with ICH GCP, suitable for inspection at any time by representatives from the Sponsor or designee and/or applicable regulatory authorities. Elements should include:

- Patient files containing informed consents and original source documents supporting entries in the eCRFs
- Study files containing the protocol with all amendments, Investigator's Brochure, copies of pre-study documentation and all correspondence to and from the EC / IRB and the Sponsor or designee
- If drug supplies are maintained at the Study Center, proof of receipt, Study Drug Product Accountability Record, Return of Study Drug Product for Destruction or confirmation of on-site destruction, final Study Drug product reconciliation, and all drug-related correspondence

All original source documents supporting entries in the eCRFs must be maintained and be readily available.

No study document should be destroyed without prior written agreement between the Sponsor and the Investigator. Should the Investigator wish to assign the study records to another party or move them to another location, he/she must notify the Sponsor.

12.4. Study Monitoring

The Sponsor representative and regulatory authority inspectors are responsible for contacting and visiting the Investigator for the purpose of inspecting the facilities and, upon request, inspecting the various records of the trial (e.g., CRFs and other pertinent data) provided that patient confidentiality is respected.

The Sponsor monitor or designee is responsible for inspecting the CRFs at regular intervals throughout the study to verify adherence to the protocol; completeness, accuracy, and consistency of the data; and adherence to local regulations on the conduct of clinical research. The monitor should have access to patient medical records and other study-related records needed to verify the entries on the CRFs.

The Investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits, including delays in completing CRFs, are resolved.

In accordance with ICH GCP and the Sponsor's audit plans, this study may be selected for audit by representatives from the Sponsor's Clinical Quality Assurance Department (or designees). Inspection of Study Center facilities (e.g., pharmacy, drug storage areas, laboratories) and review of study-related records will occur to evaluate the trial conduct and compliance with the protocol, ICH GCP, and applicable regulatory requirements.

To ensure the quality of clinical data, a clinical data management review will be performed on patient data received by the Sponsor or designee. During this review, patient data will be checked for consistency, omissions, and any apparent discrepancies. In addition, the data will be reviewed for adherence to the protocol and GCP. To resolve any questions arising from the clinical data management review process, data queries and/or Study Center notifications will be sent to the Study Center for completion and return to Sponsor or designee. All data transfers must comply with applicable law, including US Food & Drug regulations and, where applicable, the European Union's General Data Protection Regulation.

The Investigator will sign and date the indicated places on the CRF. These signatures will indicate that the Investigator inspected or reviewed the data on the CRF, the data queries, and the Study Center notifications, and agrees with the content.

12.5. Language

Case report forms must be completed in English. Generic names and trade names are acceptable for concomitant medications. Combination medications should be recorded using their trade name.

All written information and other material to be used by patients and investigative staff must use vocabulary and language that are clearly understood.

12.6. Compensation for Injury

The Sponsor maintains appropriate insurance coverage for clinical trials and will follow applicable local compensation laws. Patients will be treated and/or compensated for any study-related illness/injury in accordance with the information provided in the Compensation for Injury section of the Informed Consent document.

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14. APPENDICES

APPENDIX A. SCHEDULE OF PROCEDURES

Screening Period, Treatment Period, Early Termination from Treatment (ETFT)

Post-Treatment Period (PTP)

Appendix A Schedule of Procedures – Screening Period, Treatment Period, & Early Termination from Treatment (ETFT)

Period Name (Duration)	**Screening Period (8 Weeks)**				**Treatment Period (104 Weeks)**																												
Visit Name	**SV1**	**SV2**	**SV3**	**SV4**																												**ETFT[1]**	**Transition to PTP**
Study Week	**-9**	**-5**	**-3**	**-2**	**1**	**5**	**9**	**13**	**17**	**21**	**25[2]**	**27[2]**	**29**	**33**	**37**	**41**	**45**	**49**	**53[3]**	**57**	**61**	**65**	**69**	**73**	**77**	**81**	**85**	**89**	**93**	**97**	**101**	**105**		
Study Day	**-57**	**-29**	**-15**	**-8**	**1**	**29**	**57**	**85**	**113**	**141**	**169[2]**	**183[2]**	**197**	**225**	**253**	**281**	**309**	**337**	**365[3]**	**393**	**421**	**449**	**477**	**505**	**533**	**561**	**589**	**617**	**645**	**673**	**701**	**729**		
Visit Window (Days)	**±7**	**±7**	**±2**	**±2**	**0**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±4**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**N/A**	**N/A**
Clinic Visit[4]	X	X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X		X		X		X		X		X	X	X	
Telephone Assessment[5]																				X		X		X		X		X		X				
ISIS 702843 Administration[6]					X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Informed Consent	X																																	
Inclusion/ Exclusion	X				X																													
Medical History	X																																	
Height	X																																	
Body Weight	X				X		X		X			X		X		X			X		X		X		X		X		X		X		X	
Physical Examination[7]	X				X		X		X			X		X		X			X		X		X		X		X		X		X		X	
Vital Signs[8]	X				X[a]	X[a]	X[a]	X[a]	X[a]			X		X[a]		X[a]			X[a]		X[a]		X[a]		X[a]		X[a]		X[a]		X[a]		X	
HIV, Hepatitis B & C[9]	X																																	
Alcohol Screen	X																																	
Pregnancy Test[9,10]	X			X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
FSH[9, 11]	X																																	
Endocrine Panel[9]	X											X							X						X							X	X	
Chemistry Panel (Fasting)[9, 12, 13]	X		X		X[a]	X[a]	X[a]	X[a]	X[a]			X			X[a]		X[a]		X[a]		X[a]		X[a]		X[a]		X[a]		X[a]		X[a]		X	
Hematology[9, 13, 14]	X	X	X	X	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X	X[a]		X[a]		X[a]		X[a]		X[a]		X[a]		X[a]		X[a]		X[a]		X[a]		X	
Urinalysis[15]	X		X		X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]		X[a]		X[a]		X[a]		X[a]		X[a]		X[a]	X[a]	X	

Appendix A. Schedule of Procedures – Screening Period, Treatment Period, & Early Termination from Treatment (ETFT) Continued

Period Name (Duration)	Screening Period (8 Weeks)				Treatment Period (104 Weeks)																													
Visit Name	**SV1**	**SV2**	**SV3**	**SV4**																													**ETFT[1]**	**Transition to PTP**
Study Week	**-9**	**-5**	**-3**	**-2**	**1**	**5**	**9**	**13**	**17**	**21**	**25[2]**	**27[2]**	**29**	**33**	**37**	**41**	**45**	**49**	**53[3]**	**57**	**61**	**65**	**69**	**73**	**77**	**81**	**85**	**89**	**93**	**97**	**101**	**105**		
Study Day	**-57**	**-29**	**-15**	**-8**	**1**	**29**	**57**	**85**	**113**	**141**	**169[2]**	**183[2]**	**197**	**225**	**253**	**281**	**309**	**337**	**365[3]**	**393**	**421**	**449**	**477**	**505**	**533**	**561**	**589**	**617**	**645**	**673**	**701**	**729**		
Visit Window (Days)	**±7**	**±7**	**±2**	**±2**	**0**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±4**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**N/A**	**N/A**
Iron Metabolism Panel, including TSAT and ferritin[9, 21]	X				X[a]				X[a]			X			X[a]				X[a]				X[a]				X[a]				X[a]		X	
TSAT[9]							X[a]										X[a]				X[a]				X[a]				X[a]					
Ferritin[9]						X[a]	X[a]	X[a]		X[a]	X[a]		X[a]	X[a]		X[a]	X[a]	X[a]			X[a]				X[a]				X[a]			X[a]		
Coagulation Panel[9]	X		X		X[a]			X[a]				X							X[a]						X[a]								X	
Archived Serum Sample[9, 16]					X[a]			X[a]				X							X[a]						X[a]							X[a]	X	
ECG (12-Lead), in triplicate	X							X				X							X						X							X	X	
Echocardiogram[24]	X											X							X													X	X	
DEXA scan[24]	X																		X													X	X	
Adverse Events	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
ADA[9]					X[a]			X[a]					X[a]			X[a]			X[a]		X[a]				X[a]				X[a]				X	
Antiplatelet Antibodies[9]	X							X[a]					X[a]						X[a]						X[a]								X	
Platelet Function Testing[9, 17]		X											X[a]						X[a]						X[a]								X	
PK Blood Sampling[9]					X[b]	X[a]	X[a]	X[a]	X[a]	X[a]	X[a]	X	X[c]			X[a]			X[a]		X[a]				X[a]				X[a]			X[a]	X	
Genotypic analysis of globin genes[9]	X																																	

Appendix A. Schedule of Procedures – Screening Period, Treatment Period, & Early Termination from Treatment (ETFT) Continued

Period Name (Duration)	Screening Period (8 Weeks)				Treatment Period (104 Weeks)																													
Visit Name	**SV1**	**SV2**	**SV3**	**SV4**																													**ETFT[1]**	**Transition to PTP**
Study Week	**-9**	**-5**	**-3**	**-2**	**1**	**5**	**9**	**13**	**17**	**21**	**25[2]**	**27[2]**	**29**	**33**	**37**	**41**	**45**	**49**	**53[3]**	**57**	**61**	**65**	**69**	**73**	**77**	**81**	**85**	**89**	**93**	**97**	**101**	**105**		
Study Day	**-57**	**-29**	**-15**	**-8**	**1**	**29**	**57**	**85**	**113**	**141**	**169[2]**	**183[2]**	**197**	**225**	**253**	**281**	**309**	**337**	**365[3]**	**393**	**421**	**449**	**477**	**505**	**533**	**561**	**589**	**617**	**645**	**673**	**701**	**729**		
Visit Window (Days)	±7	±7	±2	±2	0	±7	±7	±7	±7	±7	±7	±4	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	**N/A**	**N/A**
MRI; liver, spleen, and bone marrow[18]	X											X				X			X						X								X[19]	
Ultrasound; liver and spleen[18]	X											X				X			X						X								X[19]	
EPO[9]					X[a]			X[a]				X				X[a]			X[a]		X[a]				X[a]				X[a]				X	
sTfR1[9]					X[a]			X[a]				X				X[a]			X[a]		X[a]				X[a]				X[a]				X	
GDF15[9, 20]					X[a]							X							X[a]						X[a]								X	
Erythroferrone[9]					X[a]			X[a]				X				X[a]			X[a]		X[a]				X[a]				X[a]				X	
Haptoglobin[9]					X[a]							X							X[a]						X[a]								X	
Protoporphyrin-IX[9]					X[a]							X							X[a]						X[a]								X	
Bone markers[9]					X[a]							X							X[a]						X[a]								X	
NTDT-PRO© [22]	X	X			X			X				X							X						X								X	
SF-36[22]	X	X			X							X							X						X								X	
PGIC[22]	X	X			X			X				X							X						X								X	
ISGA[22]	X	X			X							X							X						X								X	
FACIT-Fatigue[22]	X	X			X			X				X							X						X								X	
6MWT with pulse oximetry[23]	X	X			X			X				X							X						X								X	

Note: If not specifically labeled, "X" means anytime

Appendix A. Schedule of Procedures – Screening Period, Treatment Period, & Early Termination from Treatment (ETFT) Continued

Legend

[1] Patients who discontinue ISIS 702843 treatment and agree to remain in the study will attend the following clinic visits: ETFT, PTP Visit 1, PTP Visit 2, and PTP Last Visit. If patient is unable or unwilling to participate in all of these visits, the PI should determine the follow-up procedures the patient would agree to complete. Every effort should be made to complete the ETFT study procedures and observations at the time of withdrawal.

[2] The columns for Day 169 (Week 25) and Day 183 (Week 27) are outlined with bolded lines because they are associated with the first interim analysis (see Section 10.5). Note that there is no ISIS 702843 administration on Day 183 (Week 27). The regular frequency of dosing – a planned 28-day interval between each dose – is maintained throughout the Treatment Period.

[3] The column for Day 365 (Week 53) is outlined with bolded lines because it is associated with the second interim analysis (see Section 10.5).

[4] When no Clinic Visit is scheduled (SV3, SV4, and Weeks 57, 65, 73, 81, 89, and 97), urine and, if needed, blood samples may be collected at Study Clinic, at a local phlebotomy clinic, or by a home healthcare provider, at PI discretion. The testing should be done at the central laboratory except when Section 3.8 applies, in which case a local laboratory is acceptable provided that the local laboratory results are recorded by the site into the electronic database.

[5] The Study Center will conduct a phone call to check compliance and capture AEs or changes in concomitant medication usage.

[6] ISIS 702843 will be administered every 4 weeks by 1 or 2 SC injection(s) (Section 8.1). Unless the allowances in the circumstance of a public health emergency apply (Section 3.8), through Day 365 (Week 53) all doses will be administered at the Clinic, either by Study Center staff or by the trained patient/caregiver under the observation of Study Center staff. After that visit – earlier if Section 3.8 applies – self-administration at home is allowed after appropriate training of patient/caregiver, with training related to the injection step itself encouraged to start as early as Day 1 (e.g., patient or caregiver observes the injection on Day 1 and performs the injection(s) under the observation of Study Center staff at subsequent visits). In the absence of self-administration training, ISIS 702843 will continue to be administered, with the planned interval between each dose remaining 28 days, either at the Study Center by delegated staff or via home visits by Study Center staff or home healthcare providers.

[7] Full physical examination to be performed at Screening and abbreviated physical examination to be performed during the Treatment Period, as indicated, to assess changes from Screening.

[8] BP, HR, RR, temperature.

[9] Because the patients in this study have anemia, care should be taken to collect the minimum volume of blood needed to adequately support the intended test(s). Unless otherwise instructed by the PI, sampling tubes having the smallest practical volume will be used.

[10] Women who are not surgically sterile or post-menopausal. Urine test is acceptable after SV1, and is planned at time-points that do not have a scheduled blood sample for another purpose. A serum test is planned at those visits that do require a blood sample for another purpose such as Chemistry Panel or Hematology. A positive urine test is to be confirmed by a serum test.

[11] FSH is performed in all patients at all scheduled events of Endocrine Panel (see Appendix B). At SV1, FSH is also a Screening Test, with the FSH result used to address Inclusion Criterion 8 in women who are not surgically sterile, and who are potentially menopausal or post-menopausal.

[12] Fasted samples should be taken after fasting for approximately 8-10 hours. During this time the patient can drink water and they should ensure that they consume sufficient water to avoid dehydration. Fasting is not required when tests are repeated for safety reasons.

[13] If the platelet value, serum creatinine, or liver enzyme tests are uninterpretable (e.g., due to clumping, hemolysis, or quantity not sufficient) or missing, a repeat blood specimen should be re-drawn as soon as possible – ideally within 7 days, should be within 2 weeks – which may require an unscheduled visit or blood draw.

Appendix A. Schedule of Procedures – Screening Period, Treatment Period, & Early Termination from Treatment (ETFT) Continued

Legend Continued

[14] Multiple Hb measurements are required for both Screening eligibility and Baseline analysis. Both Screening Mean Hb for Eligibility and Baseline Hb for Analysis are based on at least two Hb measurements, all of which must be taken at least 6 weeks after the most recent transfusion for that patient. See Section 6.1.1.

[15] If proteinuria is observed, confirm the result and follow Section 8.6.5.

[16] Stored at -80 °C for exploration of laboratory findings, safety concerns, and/or AEs (e.g., measurement of cytokine and/or chemokine levels, measurement of additional markers of kidney function, measurement of antibodies, etc.) in this or subsequent clinical studies of ISIS 702843.

[17] It is preferred that the patient has not taken aspirin or NSAIDs (nonsteroidal anti-inflammatory drugs) throughout the 72 hours before this test, however clinical judgment by the PI should be used in deciding to withhold these drugs. Document on the ConMed CRF any use of aspirin, NSAIDS, or anti-platelet inhibitors [P2Y12 Inhibitors such as clopidogrel (Plavix), prasugrel (Effient), and ticagrelor (Brilinta)]. This test will not be performed with all patients because this test should be performed with fresh blood, and proximity to suitable equipment is not practical with some clinical sites.

[18] In regards to Inclusion Criterion 6, MRI and Ultrasound may be performed anytime within the Screening Period; however, it is preferred for these evaluations to be performed once the patient has met all other Inclusion/Exclusion Criteria, with the exception of Inclusion Criterion 5 because this criterion is evaluated based on all Hb measurements taken in the Screening Period, as described in Footnote 14, above. In the Screening Period and at all applicable time-points in the Treatment Period, MRI and Ultrasound should be conducted within one week of one another – ideally on the same day.

[19] To be done within 4 weeks of the ETFT visit.

[20] This test may not be performed, so sample will be stored frozen; if this test is performed, it may not be performed with all patients.

[21] Sampling time for serum hepcidin testing – a component of the Iron Metabolism Panel – is preferably at the same time of day from one occurrence to the next for a given patient; ideally, this consistent sampling time will be in the morning.

[22] Preferably, this survey should be completed before ISIS 702843 administration. If survey is completed after the ISIS 702843 administration at that clinic visit, this will not be considered a protocol deviation that will impact efficacy.

[23] Preferably, the 6MWT with pulse oximetry should be completed before ISIS 702843 administration. If the 6MWT with pulse oximetry is completed after the ISIS 702843 administration at that clinic visit, this will not be considered a protocol deviation that will impact efficacy.

[24] Within the Screening Period, Echocardiogram and DEXA scan may be performed anytime through SV2.

Time (in reference to ISIS 702843 administration):

[a] Pre-dose

[b] Pre-dose, and 1, 2, 4, 6 hour post-dose

[c] Pre-dose, and 3 hour post-dose

Appendix A Schedule of Procedures – Post-Treatment Period (PTP)

Period Name (Duration)	**Post-Treatment Period (13[1] Weeks)**		
Visit Name	**PTP Visit 1**	**PTP Visit 2**	**PTP Last Visit**
Week within the PTP	**PTP Week 5[2]**	**PTP Week 9**	**PTP Week 14**
Day within the PTP	**PTP Day 29[2]**	**PTP Day 57**	**PTP Day 92**
Study Day from Day 1[3]	**757**	**785**	**820**
Visit Window (Days)	**±7**	**±7**	**±7**
Clinic Visit	X	X	X
Body Weight	X		X
Physical Examination[4]	X		X
Vital Signs[5]	X		X
Pregnancy Test[6, 7]	X	X	X
Chemistry Panel (Fasting)[6, 8, 9]	X	X	X
Hematology[6, 9]	X	X	X
Urinalysis[10]	X	X	X
Iron Metabolism Panel, including TSAT and Ferritin[6, 16]	X	X	X
Coagulation Panel[6]	X[11]		
Archived Serum Sample[6, 12]	X		X
Adverse Events	X	X	X
Concomitant Medications	X	X	X
ADA[6]	X[11]	X	X
Antiplatelet Antibodies[6]	X[11]		
Platelet Function Testing[6, 15]	X		
PK Blood Sampling[6]	X	X	X
MRI; liver, spleen, and bone marrow[13]	X[11]		
Ultrasound; liver and spleen[13]	X[11]		
EPO[6]	X[11]		X
sTfR1[6]	X[11]		X
GDF15[6, 14]	X[11]		X
Erythroferrone[6]	X[11]		X
Haptoglobin[6]	X[11]		X
Protoporphyrin-IX[6]	X[11]		X
Bone markers[6]	X[11]		X
NTDT-PRO©	X[11]		X
SF-36	X[11]		X

Appendix A Schedule of Procedures – Post-Treatment Period (PTP) Continued

Period Name (Duration)	**Post-Treatment Period (13[1] Weeks)**		
Visit Name	**PTP Visit 1**	**PTP Visit 2**	**PTP Last Visit**
Week within the PTP	**PTP Week 5[2]**	**PTP Week 9**	**PTP Week 14**
Day within the PTP	**PTP Day 29[2]**	**PTP Day 57**	**PTP Day 92**
Study Day from Day 1[3]	**757**	**785**	**820**
Visit Window (Days)	**±7**	**±7**	**±7**
PGIC	X[11]		X
ISGA	X[11]		X
FACIT-Fatigue	X[11]		X
6MWT with pulse oximetry	X[11]		X

Note: If not specifically labeled, "X" means anytime

Legend

1 This includes the 4 weeks since the last dose in the Treatment Period

2 PTP Day 1 (Week 1) is equivalent in time to Day 729 (Week 105) of the Treatment Period, or the ETFT visit. There are 4 weeks between PTP Day 1 (Week 1) and PTP Visit 1

3 In this table, the values provided for "Study Day from Day 1" are only accurate for those patients who do not discontinue from ISIS 702843 treatment

4 Abbreviated physical examination to be performed during the PTP, as indicated, to assess changes from Screening

5 BP, HR, RR, temperature

6 Because the patients in this study have anemia, care should be taken to collect the minimum volume of blood needed to adequately support the intended test(s). Unless otherwise instructed by the PI, sampling tubes having the smallest practical volume will be used

7 Women who are not surgically sterile or post-menopausal. Urine test is acceptable throughout the PTP, although a serum test is planned, using a blood sample collected for another purpose such as Chemistry Panel or Hematology. A positive urine test is to be confirmed by a serum test

8 Fasted samples should be taken after fasting for approximately 8-10 hours. During this time the patient can drink water and they should ensure that they consume sufficient water in order to not become dehydrated. Fasting is not required when tests are repeated for safety reasons

9 If the platelet value, serum creatinine, or liver enzyme tests are uninterpretable (e.g., due to clumping, hemolysis, or quantity not sufficient) or missing, a repeat blood specimen should be re-drawn as soon as possible – ideally within 7 days, should be within 2 weeks – which may require an unscheduled visit or blood draw

10 If proteinuria is observed, confirm the result and follow Section 8.6.5

11 Not needed, if done at ETFT

12 Stored at -80 °C for exploration of laboratory findings, safety concerns, and/or AEs (e.g., measurement of cytokine and/or chemokine levels, measurement of additional markers of kidney function, measurement of antibodies, etc.) in this or subsequent clinical studies of ISIS 702843

13 MRI and Ultrasound should be conducted within 1 week of one another – ideally on the same day

14 This test may not be performed, so sample will be stored frozen; if this test is performed, it may not be performed with all patients

Appendix A Schedule of Procedures – Post-Treatment Period (PTP) Continued

Legend Continued

[15] It is preferred that the patient has not taken aspirin or NSAIDs (nonsteroidal anti-inflammatory drugs) throughout the 72 hours before this test, however clinical judgment by the PI should be used in deciding to withhold these drugs. Document on the ConMed CRF any use of aspirin, NSAIDS, or anti-platelet inhibitors [P2Y12 Inhibitors such as clopidogrel (Plavix), prasugrel (Effient), and ticagrelor (Brilinta)]. This test will not be performed with all patients because this test should be performed with fresh blood, and proximity to suitable equipment is not practical with some clinical sites

[16] Sampling time for serum hepcidin testing – a component of the Iron Metabolism Panel – is preferably at the same time of day from one occurrence to the next for a given patient; ideally, this consistent sampling time will be in the morning

APPENDIX B. List of Laboratory Tests

Appendix B List of Laboratory Tests

Based on emerging data from this or future studies, additional tests not listed below may be performed on stored samples to better characterize the profile of ISIS 702843 or other similar oligonucleotides.

Clinical Chemistry Panel
- Calcium[1]
- Phosphorus[1]
- Alkaline phosphatase (ALP)[1]
- Sodium
- Potassium
- Chloride
- Bicarbonate
- Total protein
- Albumin
- Magnesium
- Glucose (FBS)
- BUN
- Creatinine
- Uric acid
- Total bilirubin
- Direct (conjugated) bilirubin
- Indirect (unconjugated) bilirubin
- ALT
- AST
- Creatinine kinase
- GGT
- LDH

Bone Markers
- Calcium[1]
- Phosphorus[1]
- ALP[1]
- Parathyroid hormone
- Vitamin D

Screening Tests
- Hepatitis B surface antigen
- Hepatitis C antibody
- HIV antibody
- FSH[2]
- Serum βhCG
- Alcohol Screen

Coagulation Panel
- PT
- INR
- aPTT
- Antithrombin III
- Protein S
- Protein C

Iron Metabolism Panel
- Serum hepcidin
- Serum iron
- Serum transferrin
- Serum ferritin
- TSAT
- NTBI

Platelet Count Safety Panel[4]
- Peripheral smear
- Fibrinogen split products or D-dimer on fresh blood
- Citrated sample for platelets
- Coagulation panel (PT/INR, aPTT)
- CBC with reticulocytes and mean platelet volume
- Total globulins: IgG and IgM
- Complement: total C3, total C4, C5a
- Serology for hepatitis B virus, HCV, HIV (if not done recently for Screening)
- Antiplatelet antibodies
- ADA

Hematology Panel
- RBC count
- Hb
- Hematocrit
- RDW
- MCV, MCH, MCHC
- Erythroblast count
- Reticulocyte count
- Platelet count
- White blood cell count
- WBC differential (% and absolute)
- Peripheral blood smear
- CHr[3]

Endocrine Panel
- TSH
- Free T4
- Free T3
- FSH[2]
- LH
- Testosterone (men only)

Pharmacokinetics
- ISIS 702843 levels in plasma[5]

Immunogenicity
- Anti-ISIS 702843 antibodies (i.e., anti-drug antibodies [ADA])[5]

Urinalysis
- Color
- Appearance
- Specific gravity
- pH
- UPCR
- Protein
- Blood
- Ketones
- Urobilinogen
- Glucose
- Bilirubin
- Leukocyte esterase
- Nitrate
- Microscopic examination[6]

Other
- EPO
- sTfR1
- GDF15
- Erythroferrone
- Haptoglobin
- Protoporphyrin-IX
- Platelet function testing
- Genotypic analysis of globin genes (included in the genetic assessment of a panel of blood-related disorders)

Appendix B. List of Laboratory Tests Continued

Legend

[1] Calcium, Phosphorus, and ALP are performed at all scheduled events of Clinical Chemistry Panel. At a subset of these events, Bone Markers are also evaluated. At these time-points, the Calcium, Phosphorus, and ALP results from Clinical Chemistry Panel are also applicable to Bone Markers.

[2] FSH is performed in all patients at all scheduled events of Endocrine Panel. At SV1, FSH is also a Screening Test, with the FSH result used to address Inclusion Criterion 8 in women who are not surgically sterile, and who are potentially menopausal or post-menopausal.

[3] Either traditional CHr or "reticulocyte hemoglobin equivalent" (i.e., run on Sysmex machines), ideally being consistent with respect to which type of machine is used intra-patient, and reporting as percent change

[4] These tests should be done if a patient has a platelet count < 50,000/mm^3 (Section 8.5.2).

[5] Plasma samples may also be used for profiling of drug binding proteins, bioanalytical method validation purposes, stability assessments, metabolite assessments, immunogenicity testing (or possibly for purposes of immunogenicity assay development and/or validation), or to assess other actions of ISIS 702843 with plasma constituents.

[6] Will be performed on abnormal findings unless otherwise specified.

Appendix C. PK Sampling Schedule

Treatment Period and Early Termination from Treatment (ETFT)

Post-Treatment Period (PTP)

Appendix C PK Sampling Schedule

Treatment Period and Early Termination from Treatment (ETFT)

Time Points for PK Blood Draws															
Week 1	**Week 5**	**Week 9**	**Week 13**	**Week 17**	**Week 21**	**Week 25**	**Week 27**	**Week 29**	**Week 41**	**Week 53**	**Week 61**	**Week 77**	**Week 93**	**Week 105**	**ETFT**
Day 1	**Day 29**	**Day 57**	**Day 85**	**Day 113**	**Day 141**	**Day 169**	**Day 183**	**Day 197**	**Day 281**	**Day 365**	**Day 421**	**Day 533**	**Day 645**	**Day 729**	
Pre-dose, and 1, 2, 4, and 6 hour post-dose	Pre-dose	Pre-dose	Pre-dose	Pre-dose	Pre-dose	Pre-dose	Anytime	Pre-dose and 3-hour post-dose	Pre-dose	Pre-dose	Pre-dose	Pre-dose	Pre-dose	Pre-dose	Anytime
Number of PK Blood Draws															
5	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1

Note: Time is shown as hours relative to dose of ISIS 702843

Post-Treatment Period (PTP)

Time Points for PK Blood Draws		
PTP Visit 1	**PTP Visit 2**	**PTP Last Visit**
PTP Week 5	**PTP Week 9**	**PTP Week 14**
PTP Day 29	**PTP Day 57**	**PTP Day 92**
Anytime	Anytime	Anytime
Number of PK Blood Draws		
1	1	1

APPENDIX D. Grading Scale For Adverse Events Relating To Laboratory Abnormalities

Appendix D Grading Scale for Adverse Events Relating to Laboratory Abnormalities

The following grading recommendations for adverse events relating to laboratory test abnormalities and adverse events at the injection site are based on the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0, November 2017. The severity of AEs and SAEs relating to laboratory tests and adverse events at the injection site may be graded based on these criteria.

Alternatively, the Investigator should classify events and laboratory findings as mild, moderate, or severe based on the clinical significance of the event and laboratory finding for that patient, for example, laboratory findings related to Hb, ALT, and AST.

Any AE not listed below will be graded as described in Section 9.4.3.2

Adverse Event	Mild	Moderate	Severe
Hematology			
aPTT prolonged	>ULN - 1.5 x ULN	>1.5 - 2.5 x ULN	>2.5 x ULN; bleeding
Eosinophils increased[1]	>ULN and >Baseline	-	Steroids Initiated
Fibrinogen decreased	<1.0 - 0.75 x LLN; if abnormal, <25% decrease from baseline	<0.75 - 0.5 x LLN; if abnormal, 25 - <50% decrease from baseline	<0.5 x LLN; if abnormal, ≥50% decrease from baseline
Hemoglobin decreased (Anemia)	Hemoglobin (Hgb) <LLN - 10.0 g/dL; <LLN - 6.2 mmol/L; <LLN - 100 g/L	Hgb <10.0 - 8.0 g/dL; <6.2 - 4.9 mmol/L; <100 - 80g/L	Hgb <8.0 g/dL; <4.9 mmol/L; <80 g/L; transfusion indicated
Hemoglobin increased**	Increase in >0 - 2 g/dL above ULN or above baseline if baseline is above ULN	Increase in >2 - 4 g/dL above ULN or above baseline if baseline is above ULN	Increase in >4 g/dL above ULN or above baseline if baseline is above ULN
INR increased	>1.2 - 1.5; >1 - 1.5 times above baseline if on anticoagulation	>1.5 - 2.5; >1.5 - 2.5 x baseline if on anticoagulation; monitoring only indicated	>2.5; >2.5 x baseline if on anticoagulation; dose adjustment indicated
Lymphocyte count decreased	<LLN - 800/mm^3; <LLN - 0.8 x 10^9/L	<800 - 500/mm^3; <0.8 - 0.5 x 10^9 /L	<500 /mm^3; <0.5 x 10^9 /L
Lymphocyte count increased	-	>4000/mm^3 - 20,000/mm^3	>20,000/mm^3
Neutrophil count decreased	<LLN - 1500/mm^3; <LLN - 1.5 x 10^9 /L	<1500 - 1000/mm^3; <1.5 - 1.0 x 10^9 /L	<1000/mm^3; <1.0 x 10^9 /L
Platelet count decreased	<LLN - 75,000/mm^3; <LLN - 75.0 x 10^9 /L	<75,000 - 50,000/mm^3; <75.0 - 50.0 x 10^9 /L	<50,000/mm^3; <50.0 x 10^9 /L
White blood cell decreased	<LLN - 3000/mm^3; <LLN - 3.0 x 10^9 /L	<3000 - 2000/mm^3; <3.0 - 2.0 x 10^9 /L	<2000/mm^3; <2.0 x 10^9 /L
Chemistry			
Acidosis	pH <normal, but >=7.3	-	pH <7.3
Alanine aminotransferase increased	>ULN - 3.0 x ULN if baseline normal 1.5 - 3.0 x baseline if baseline abnormal	>3.0 - 5.0 x ULN if baseline normal >3.0 - 5.0 x baseline if baseline abnormal	>5.0 x ULN if baseline normal >5.0 x baseline if baseline abnormal
Alkaline phosphatase increased	>ULN - 2.5 x ULN if baseline normal 2.0 - 2.5 x baseline if baseline abnormal	>2.5 - 5.0 x ULN if baseline normal >2.5 - 5.0 x baseline if baseline abnormal	>5.0 x ULN if baseline normal >5.0 x baseline if baseline was abnormal
Alkalosis	pH >normal, but ≤7.5	-	pH >7.5
Aspartate aminotransferase increased	>ULN - 3.0 x ULN if baseline normal 1.5 - 3.0 x baseline if baseline abnormal	>3.0 - 5.0 x ULN if baseline normal >3.0 - 5.0 x baseline if baseline abnormal	>5.0 x ULN if baseline normal >5.0 x baseline if baseline abnormal
Blood bilirubin increased	>ULN - 1.5 x ULN if baseline normal >1.0 - 1.5 x baseline if baseline abnormal	>1.5 - 3.0 x ULN if baseline normal >1.5 - 3.0 x baseline if baseline abnormal	>3.0 x ULN if baseline normal >3.0 x baseline if baseline abnormal
Cardiac troponin I increased	Levels above the upper limit of normal and below the level of myocardial infarction as defined by the manufacturer	-	Levels consistent with myocardial infarction as defined by the manufacturer

Appendix D. Grading Scale For Adverse Events Relating To Laboratory Abnormalities Continued

Adverse Event	Mild	Moderate	Severe
Cardiac troponin T increased	Levels above the upper limit of normal and below the level of myocardial infarction as defined by the manufacturer	-	Levels consistent with myocardial infarction as defined by the manufacturer
CD4 lymphocytes decreased	<LLN - 500/mm^3; <LLN - 0.5 x 10^9 /L	<500 - 200/mm^3; <0.5 - 0.2 x 10^9 /L	<200/mm^3; <0.2 x 10^9 /L
CPK increased*	>ULN - <6 ULN	6 - 10 x ULN	>10 x ULN
Creatinine increased**	>ULN - 1.5 x ULN if baseline normal > 1.0 - 1.5 x baseline if baseline abnormal	>1.5 - 3.0 x ULN if baseline normal >1.5 - 3.0 x baseline if baseline abnormal	>3.0 x ULN if baseline normal >3.0 x baseline if baseline abnormal
GGT increased	>ULN - 2.5 x ULN if baseline normal 2.0 - 2.5 x baseline if baseline abnormal	>2.5 - 5.0 x ULN if baseline normal >2.5 - 5.0 x baseline if baseline abnormal	>5.0 x ULN if baseline normal >5.0 x baseline if baseline abnormal
Hypercalcemia	Corrected serum calcium of >ULN - 11.5 mg/dL; >ULN - 2.9 mmol/L; Ionized calcium >ULN - 1.5 mmol/L	Corrected serum calcium of >11.5 - 12.5 mg/dL; >2.9 - 3.1 mmol/L; Ionized calcium >1.5 - 1.6 mmol/L; symptomatic	Corrected serum calcium of >12.5 mg/dL; >3.1 mmol/L; Ionized calcium >1.6 mmol/L; hospitalization indicated
Hyperglycemia††	Fasting glucose value ≥126 mg/dL (7.0 mmol/L)	Change in daily management to maintain fasting blood glucose <126 mg/dL (7.0 mmol/L); e.g. addition of oral antiglycemic agent; workup for diabetes	Insulin therapy initiated; hospitalization indicated
Hyperkalemia	>ULN - 5.5 mmol/L	>5.5 - 6.0 mmol/L; intervention initiated	>6.0; hospitalization indicated
Hypermagnesemia	>ULN - 3.0 mg/dL; >ULN - 1.23 mmol/L	-	>3.0 mg/dL; >1.23 mmol/L
Hypernatremia	>ULN - 150 mmol/L	>150 - 155 mmol/L; intervention initiated	>155 mmol/L; hospitalization indicated
Hyperphosphatemia	Laboratory finding only and intervention not indicated	Noninvasive intervention indicated	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated
Hyperuricemia	>ULN without physiologic consequences	-	>ULN with physiologic consequences
Hypoalbuminemia	<LLN - 3 g/dL; <LLN - 30 g/L	<3 - 2 g/dL; <30 - 20 g/L	<2 g/dL; <20 g/L
Hypocalcemia	Corrected serum calcium of <LLN - 8.0 mg/dL; <LLN - 2.0 mmol/L; Ionized calcium <LLN - 1.0 mmol/L	Corrected serum calcium of <8.0 - 7.0 mg/dL; <2.0 - 1.75 mmol/L; Ionized calcium <1.0 - 0.9 mmol/L; symptomatic	Corrected serum calcium of <7.0 mg/dL; <1.75 mmol/L; Ionized calcium <0.9 mmol/L; hospitalization indicated
Hypoglycemia‡	≥54 mg/dL - <70 mg/dL ≥3.0 mmol/L - <3.9 mmol/L	<54 mg/dL (3.0 mmol/L) AND no assistance required to actively administer carbohydrates, glucagon, or take other corrective actions	Requires assistance of another person to actively administer carbohydrates, glucagon, or take other corrective actions
Hypokalemia	<LLN - 3.0 mmol/L	symptomatic with <LLN - 3.0 mmol/L; intervention indicated	<3.0 mmol/L; hospitalization indicated
Hypomagnesemia	<LLN - 1.2 mg/dL; <LLN - 0.5 mmol/L	<1.2 - 0.9 mg/dL; <0.5 - 0.4 mmol/L	<0.9 mg/dL; <0.4 mmol/L
Hyponatremia	<LLN - 130 mmol/L	125-129 mmol/L and asymptomatic	125-129 mmol/L symptomatic; 120-124 mmol/L regardless of symptoms
Hypophosphatemia	Laboratory finding only and intervention not indicated	Oral replacement therapy indicated	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of existing hospitalization indicated
Lipase increased	>ULN - 1.5 x ULN	>1.5 - 2.0 x ULN; >2.0 - 5.0 x ULN and asymptomatic	>2.0 x ULN with signs or symptoms
Serum amylase increased	>ULN - 1.5 x ULN	>1.5 - 2.0 x ULN; >2.0 - 5.0 x ULN and asymptomatic	>2.0 x ULN with signs or symptoms

Appendix D. Grading Scale For Adverse Events Relating To Laboratory Abnormalities Continued

Adverse Event	Mild	Moderate	Severe
Urine			
Proteinuria Adults	1+ proteinuria; urinary protein ≥ULN - <1.0 g/24 hrs	2+ and 3+ proteinuria; urinary protein 1.0 - 3.4 g/24 hrs;	4+ proteinuria; Urinary protein ≥3.5 g/24 hrs;
Children	-	Urine P/C (Protein/Creatinine) ratio 0.5 - 1.9	Urine P/C >1.9
Hematuria	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; urinary catheter or bladder irrigation indicated	Gross hematuria; transfusion, IV medications or hospitalization indicated; elective invasive intervention indicated
Adverse Events at the Injection Site			
Adverse events at the injection site**	An event at the injection site (e.g. erythema, tenderness, itching) that is easily tolerated by the subject and does not affect the subject's usual daily activities	- Persistent (>24 hours) pain, phlebitis or edema; OR - Lipodystrophy, hair growth or alopecia, OR - Prolonged (>1 month) hypo/hyperpigmentation	- Ulceration or necrosis; severe tissue damage; operative intervention indicated, OR - Any event at the injection site that is incapacitating

†Grading for this parameter is derived from the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials, Sept 2007

*Grading for this parameter is derived from the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events Version 2.0, Nov 2014

††Modified for consistency with ADA "Standards of Medical Care in Diabetes - 2018" Diabetes Care 2018;41(Suppl. 1):S13–S27. https://doi.org/10.2337/dc18-S002

‡Modified for consistency with ADA "Glycemic Targets: Standards of Medical Care in Diabetes - 2018", Diabetes Care 2018;41(Suppl. 1):S55–S64. https://doi.org/10.2337/dc18-S006

**Adapted from the original CTCAE V5.0 scale

APPENDIX E. Measures of Quality of Life & Functional Status of the Patient

NTDT-PRO©

SF-36

PGIC

ISGA

FACIT-Fatigue

6MWT

Appendix E Measures of Quality of Life & Functional Status of the Patient

NTDT-PRO© ("Non-transfusion Dependent Thalassemia-Patient Reported Outcome©)

The following NTDT-PRO© has been published – see 2 citations below – and is used by permission.

- Development of a patient-reported outcomes symptom measure for patients with nontransfusion-dependent thalassemia (NTDT-PRO©).

  (Taher, Viprakasit, et al. 2019)

- Validation of a patient-reported outcomes symptom measure for patients with nontransfusion-dependent thalassemia (NTDT-PRO©).

  (Taher, Cappellini, et al. 2019)

NTDT-PRO©.v2 (version 2) Instrument

For each of the following questions, please choose the number that best describes the symptoms that you may have experienced during the past 24 hours.

1. How would you rate your tiredness (lack of energy) when you were not doing physical activity during the past 24 hours?

☐ 0 No tiredness ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Extreme tiredness

2. How would you rate your tiredness (lack of energy) when you were doing physical activity during the past 24 hours?

☐ 0 No tiredness ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Extreme tiredness

3. How would you rate your weakness (lack of strength) when you were not doing physical activity during the past 24 hours?

☐ 0 No weakness ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Extreme weakness

4. How would you rate your weakness (lack of strength) when you were doing physical activity during the past 24 hours?

☐ 0 No weakness ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☐ 7 ☐ 8 ☐ 9 ☐ 10 Extreme weakness

Appendix E: Measures of Quality of Life & Functional Status of the Patient Continued

NTDT-PRO Continued

5. How would you rate your shortness of breath when you were not doing physical activity during the past 24 hours?

☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
0	1	2	3	4	5	6	7	8	9	10
No shortness of breath										Extreme shortness o breath

6. How would you rate your shortness of breath when you were doing physical activity during the past 24 hours?

☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
0	1	2	3	4	5	6	7	8	9	10
No shortness of breath										Extreme shortness of breath

For validation purposes only

7. During the past 24 hours, how would you rate the overall severity of your thalassemia symptoms?

☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
0	1	2	3	4	5	6	7	8	9	10
No symptoms										Very severe symptoms

For validation purposes only (administered once every 3 weeks)

8. How would you rate the overall change in your thalassemia symptoms since the start of this study?

□ A great deal better

□ Much better

□ A little better

□ No change

□ A little worse

□ Much worse

□ A great deal worse

Appendix E: Measures of Quality of Life & Functional Status of the Patient Continued

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

Appendix E: Measures of Quality of Life & Functional Status of the Patient Continued

SF-36 Continued

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

Appendix E: Measures of Quality of Life & Functional Status of the Patient Continued

SF-36 Continued

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

Appendix E: Measures of Quality of Life & Functional Status of the Patient Continued

SF-36 Continued

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

Appendix E: Measures of Quality of Life & Functional Status of the Patient Continued

SF-36 **Continued**

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

Appendix E: Measures of Quality of Life & Functional Status of the Patient Continued

SF-36 Continued

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 E: Measures of Quality of Life & Functional Status of the Patient Continued

Patient Global Impression of Change (PGIC)

PATIENT GLOBAL IMPRESSION OF CHANGE (PGIC)

Since the start of the study, my overall status is:

✓ one box only:

(1) ☐ Very Much Improved

(2) ☐ Much Improved

(3) ☐ Minimally Improved

(4) ☐ No Change

(5) ☐ Minimally Worse

(6) ☐ Much Worse

(7) ☐ Very Much Worse

(US/English)

Appendix E: Measures of Quality of Life & Functional Status of the Patient Continued

Investigator's Static Global Assessment (ISGA)

Instructions:

*Assess the patient **at the current time** using the category of disease status below and provide the corresponding ISGA score.*

ISGA SCORE for β-Thalassemia Intermedia	**Current Status of Disease**
0	No Signs or Symptoms
1	Minimal Signs and Symptoms
2	Mild Signs and Symptoms
3	Moderate Signs and Symptoms
4	Severe Signs and Symptoms

Appendix E: Measures of Quality of Life & Functional Status of the Patient Continued

Functional Assessment of Chronic Illness Therapy – Fatigue (FACIT-Fatigue)

FACIT Fatigue Scale (Version 4)

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

		Not at all	A little bit	Some-what	Quite a bit	Very much
HI7	I feel fatigued	0	1	2	3	4
HI12	I feel weak all over	0	1	2	3	4
An1	I feel listless ("washed out")	0	1	2	3	4
An2	I feel tired	0	1	2	3	4
An3	I have trouble starting things because I am tired	0	1	2	3	4
An4	I have trouble finishing things because I am tired	0	1	2	3	4
An5	I have energy	0	1	2	3	4
An7	I am able to do my usual activities	0	1	2	3	4
An8	I need to sleep during the day	0	1	2	3	4
An12	I am too tired to eat	0	1	2	3	4
An14	I need help doing my usual activities	0	1	2	3	4
An15	I am frustrated by being too tired to do the things I want to do	0	1	2	3	4
An16	I have to limit my social activity because I am tired	0	1	2	3	4

Appendix E: Measures of Quality of Life & Functional Status of the Patient Continued

6-Minute Walk Test (6MWT) - Borg CR10 Scale for Dyspnea and Fatigue

Instruction. Use this rating scale to report how strong your perception is. It can be exertion, pain or something else. First look at the verbal expressions. Start with them and then the numbers. Of these ten (10) or "Extremely strong", "Maximal" is a very important intensity level. This is the most intense perception or feeling you have ever had.

If your experience or feeling is "Very weak", you should say "1", if it is "Moderate", say "3". Note that "Moderate" is "3" and thus weaker than "Medium", "Mean" or "Middle". If the experience is "Strong" or "Heavy" (it feels "Difficult") say "5". Note that "Strong" is about half of "Maximal". If your feeling is "Very strong", choose a number from 6 to 8. If your perception or feeling is stronger than "10", - "Extremely strong", "Maximal" – you can use a larger number, e.g. 12 or still higher (that's why "Absolute maximum" is marked with a dot "•").

It's very important that you report what you actually experience or feel, not what you think you should report. Be as spontaneous and honest as possible and try to avoid under- or overestimating. Look at the verbal descriptors and then choose a number.

When rating exertion give a number that corresponds to how hard and strenuous you perceive the work to be. The perception of exertion is mainly felt as strain and fatigue in your muscles and as breathlessness or any aches.

0 "Nothing at all", means that you don't feel any exertion whatsoever, no muscle fatigue, no breathlessness or difficulties breathing.

1 "Very weak" means a very light exertion. As taking a shorter walk at your own pace.

3 "Moderate" is somewhat but not especially hard. It feels good and not difficult to go on.

5 "Strong". The work is hard and tiring, but continuing isn't terribly difficult. The effort and exertion is about half as intense as "Maximal".

7 "Very strong" is quite strenuous. You can still go on, but you really have to push yourself and you are very tired.

10 "Extremely strong – Maximal" is an extremely strenuous level. For most people this is the most strenuous exertion they have ever experienced previously in their lives.

● Is "Absolute maximum" for example "12" or even more.

Any questions?

Borg CR10 scale®
© G. Borg, 1998, 2007
English

Appendix E: Measures of Quality of Life & Functional Status of the Patient Continued

6MWT Continued – Borg CR10 Scale for Dyspnea and Fatigue Continued

0	Nothing at all	
0.3		
0.5	Extremely weak	Just noticeable
0.7		
1	Very weak	
1.5		
2	Weak	Light
2.5		
3	Moderate	
4		
5	Strong	Heavy
6		
7	Very strong	
8		
9		
10	Extremely strong	"Maximal"
11		
↑		
●	Absolute maximum	Highest possible

Borg CR10 Scale®
© Gunnar Borg, 1982, 1998, 2004
English

Protocol

Version:	1
Version Date:	17 May 2021
Title:	Protocol Amendment 2: A Phase 2a, Randomized, Open-Label Study to Evaluate the Efficacy, Safety, Tolerability, PK and PD of ISIS 702843 Administered SC to Patients with Non-Transfusion Dependent β-Thalassemia Intermedia

[redacted]

Statistical Analysis Plan

ISIS 702843-CS2

A Phase 2a, Randomized, Open-Label Study to Evaluate the Efficacy, Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of ISIS 702843 Administered Subcutaneously to Patients with Non-Transfusion Dependent β-Thalassemia Intermedia

Date: 22 May 2023

Version: 3.0

SIGNATURES

Ionis Pharmaceuticals, Inc.
2855 Gazelle Court Carlsbad, CA 92010

Compound Name: TMPRSS6-L (ISIS 702843) Sapablursen

Protocol: ISIS 702843-CS2

Study Title: A Phase 2a, Randomized, Open-Label Study to Evaluate the Efficacy, Safety, Tolerability, Pharmacokinetics and Pharmacodynamics of ISIS 702843 Administered Subcutaneously to Patients with Non-Transfusion Dependent β-Thalassemia Intermedia

Issue Date: 22 May 2023

[redacted]

Table of Contents

REVISION HISTORY

Version No.	Effective Date	Summary of Changes
V1.0	30 Jun 2020	The original SAP.
V1.1	13 Jul 2020	Removed the signature page and updated the page number. The sign-off process was conducted via Veeva system.
V2.0	23 Jul 2021	1. Updated Section 1.1 to incorporate changes of dosing amount, randomization, enrolment, and sample size per Protocol Amendment 2. 2. Changed the section number of Safety Objectives and Safety Endpoints to match the order with protocol. 3. Updated Section 2.2 to clarify the randomization scheme in the original Protocol, Protocol Amendment 1 and 2. Specified that the dosing amount increased to 120 mg per dose for Cohort A and B, and to 120 mg per dose for 3 consecutive doses, followed by 160 mg per dose for Cohort C. 4. Updated Section 3.1 to specify the total number of patients enrolled changed from 36 to 24. 12 patients will be enrolled for Cohort A and B; and 12 patients will be enrolled for Cohort C. 5. Updated Section 3.2.7 to add 6-minute walk test with Pulse Oximetry. 6. Updated Section 3.3.2 to change the number of patients required for interim analysis per Protocol Amendment 2. 7. Updated Section 3.3.3 to clarify the changes of enrollment process per Protocol Amendment 2. 8. Updated Section 3.4 to add analytical considerations regarding Hb measurements. 9. Updated Section 3.7.3 to add analytical considerations for local lab performed due to COVID-19 public health emergency. 10. Removed the description related to COVID-19 public health emergency in Section 3.2.1. Rephrased the description and added it in Section 3.2.5. 11. Added Section 3.2.5 and 3.2.6 to provide more analytical considerations for COVID-19 public health emergency. Reorganized the section number of Section 3.2.5 – 3.2.8. 12. Added the list of parameters used for correlation analysis and specify the details of time-to-event analysis in Section 3.5. 13. Added Section 2.5.4 to clarify the details of Anti-Drug Antibody sample data. 14. Added Section 3.7.8, 3.7.9 and 3.7.10 to specify analytical considerations regarding Ultrasound, MRI, Leg Ulcer, and Genetic sample. 15. Added imputation rules for missing/partial date of adverse events and prior/concomitant medications.

Version No.	Effective Date	Summary of Changes
V3.0	18 May 2023	1. Updated the whole document with the new SAP template. 2. Added the Abbreviation Section. 3. Updated Section 1.3 Endpoints to remove the exploratory endpoints listed below for the final analyses, because the Sponsor terminated the study due to a lack of efficacy and the CSR will be abbreviated: • Change from baseline of erythroblast count • Change from baseline of spleen iron concentration, and non-transferrin bound iron (NTBI) • Change from baseline of plasma haptoglobin, plasma protoporphyrin IX, and blood smears • Change from baseline of antithrombin III, Protein S, and Protein C • Change from baseline on quality of life measures, including change from baseline in NTDT-PRO©, SF-36, PGIC, ISGA, and FACIT-Fatigue • Change from baseline on functional status of patient using 6MWT instrument 4. Updated Section 1.2 Objectives. 5. Updated Section 3.2.1 Statistical Design Summary, COVID-19 related information is in section COVID-19 Related Impact within Section 3.2.1 instead of Section 3.2.6. 6. Moved Section 3.2.3 Disposition of Subjects, Section 3.2.4 Demographic and Baseline Characteristics, and Section 3.2.5 COVID-19 Related Impact to Section 3.2.1 Statistical Design Summary. 7. Moved Section 3.2.6 Handling of Missing Data to Section 3.7.1 Adverse Events. 8. Removed Section 3.2.7 Measures of Questionnaires from the Quality of Life (QoL) and Functional Status. 9. Updated Section 3.3.2 Analysis of Primary Endpoint to remove the description of potential additional comparison between cohorts. 10. Updated Section 3.3.4 Planned Interim Analyses to clarify that formal interim analyses were not performed. 11. Updated Section 3.5 Exploratory Analyses to remove correlation analysis, time to event analysis, and QoL related analysis. 12. Updated Section 3.5 Exploratory Analyses to clarify some exploratory endpoints will not be analyzed. 13 Updated Section 3.7 Safety Analyses to remove Echocardiogram and DEXA, Ultrasound and MRI assessment for spleen, and bone marrow, and Leg Ulcers. 14. Updated Section 3.7.4 Exposure and Drug Administration to add additional exposure summary.

ABBREVIATIONS

Abbreviation	Definition
ADA	anti-drug antibodies
AE	adverse event
ALT	alanine aminotransferase (formerly referred to as serum glutamic pyruvic transaminase [SGPT])
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase (formerly referred to as serum glutamic oxaloacetic transaminase [SGOT])
CHr	reticulocyte hemoglobin content
CRF	case report form
CSR	clinical study report
DEXA	dual energy X-ray absorptiometry
ECG	electrocardiogram
eCRF	electronic Case Report Form
EDC	electronic data capture
EPO	erythropoietin
ETFT	early termination from treatment
FACIT	Functional Assessment of Chronic Illness Therapy
FAS	Full Analysis Set
Fe/g	iron (Fe) per gram of dry weight of liver
GCP	Good Clinical Practice
GDF15	growth differentiation factor 15
Hb	hemoglobin
ICH	International Conference on Harmonization
IE	ineffective erythropoiesis
INR	international normalized ratio
IRT	Interactive Response Technology
ISGA	Investigator's Static Global Assessment
ISIS 702843	antisense inhibitor of TMPRSS6
LDH	lactate dehydrogenase
LIC	liver iron concentration
MCH	mean corpuscular hemoglobin (amount)
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MedDRA™	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
NTBI	non-transferrin-bound iron
NTDT	non-transfusion dependent thalassemia
on study	The patient is 'on study' from signing of the informed consent until their last study visit
PD	pharmacodynamic(s)
PGIC	Patient Global Impression of Change
PHT	pulmonary hypertension

Abbreviation	Definition
PI	Principal Investigator
PK	pharmacokinetic(s)
PRO	patient-reported outcome(s)
PT	prothrombin time
PTP	post-treatment period
RBC	red blood cell(s)
SAE	serious adverse event
SAP	Statistical Analysis Plan
SC	subcutaneous(ly)
SOC	System Organ Classes
sTfR1	soluble transferrin receptor 1
Study Day 1	defined as the first day ISIS 702843 is administered to the patient
Study Drug	ISIS 702843 (there is no placebo in this study)
TEAE	treatment-emergent adverse event
TMPRSS6	transmembrane protease, serine 6
TSAT	transferrin saturation
ULN	upper limit of normal

1.0 INTRODUCTION

This document provides a description of the statistical methods and procedures to be implemented for the analyses of data from Ionis Pharmaceuticals, Inc. study with Protocol Number ISIS 702843-CS2. Section 1 discusses study design, objectives, and endpoints; Section 2 provides the study procedures; Section 3 provides the detailed plan for the statistical analyses.

The purpose of this plan is to provide specific guidelines from which the analysis will proceed. Any deviations from these guidelines will be documented in the clinical study report (CSR). Within this document, the terms 'patient' and 'subject' are both used to describe the individual who enrolls in this study.

1.1 Study Overview

This is a Phase 2a, open-label, randomized, multi-center, multi-region study of subcutaneously (SC) administered ISIS 702843 in patients with non-transfusion dependent β-Thalassemia Intermedia.

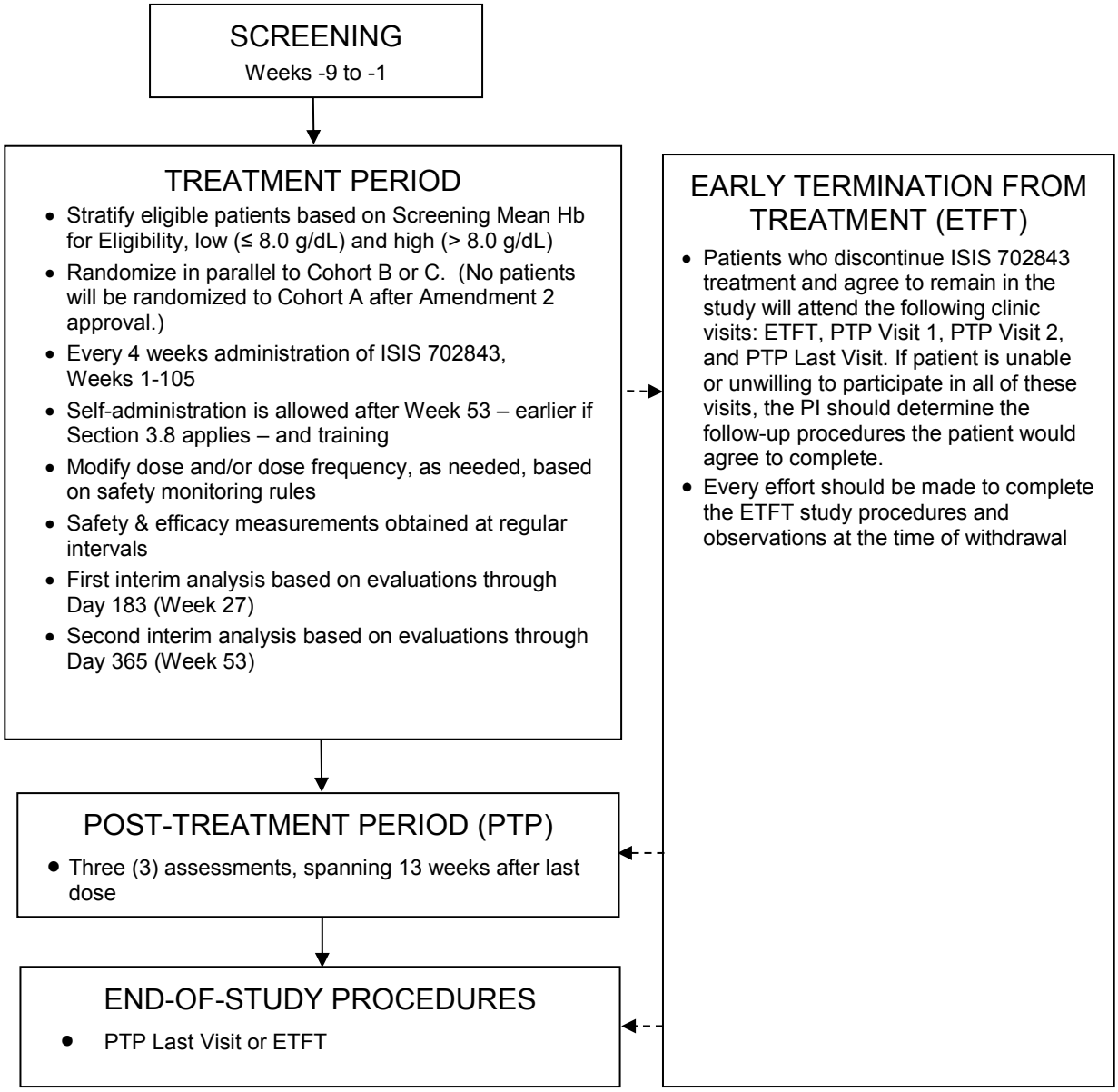
The study will comprise 3 cohorts: Cohorts A, B, and C. Under the original Protocol and Protocol Amendment 1, the study was to enroll approximately 36 patients, randomized to Cohorts A (ISIS 702843 30 mg), B (ISIS 702843 50 mg), and C (ISIS 702843 80 mg) in a ratio of 1:1:1.

Upon the approval of Protocol Amendment 2, the study design was updated, and the patients were randomized in parallel to Cohort B or Cohort C, with no new patients randomized to Cohort A. In addition, the dose level for Cohorts A and B became the same at 120 mg ISIS 702843 per dose. The dose level for Cohort C became 120 mg ISIS 702843 per dose for three consecutive doses, followed by 160 mg ISIS 702843 per dose, if dose escalation was allowed based on a demonstration of adequate safety. The eligible patients were stratified based on Screening mean Hb status of low (≤ 8.0 g/dL) or high (> 8.0 g/dL).

There were 12 patients in Cohorts A and B combined, and 17 patients in Cohort C. The study consisted of Screening, Treatment, and Post-Treatment periods. The overall length of a subject's participation was to be approximately 29 months (up to 2 months for the Screening Period, a 24-month Treatment Period, and a 3-month Post-Treatment Period).

Each patient was to be treated for up to 2 years, receiving up to 27 doses of ISIS 702843, with a planned 28-day interval between each dose. [redacted] Unless the allowances in the circumstance of a public health emergency apply (Section 3.8 in the protocol), all Study Drug injections will be SC administered at the clinic through Day 365 (Week 53) and can be SC administered away from the clinic after that visit.

The study design and treatment schema are depicted as follows:



1.2 Objectives

1.2.1 Primary Objective

Evaluate the efficacy of antisense inhibitor of TMPRSS6 (ISIS 702843) by demonstrating an improvement in hemoglobin (Hb) concentration at Week 27 of treatment.

1.2.2 Secondary Objectives

- Evaluate the efficacy of ISIS 702843 by demonstrating an improvement (increase) in Hb concentration at Week 53 of treatment.
- Evaluate the efficacy of ISIS 702843 by demonstrating an improvement (decrease) in liver iron concentration (LIC) at Week 53 of treatment.

1.2.3 Exploratory Objectives

- Evaluate the impact of ISIS 702843 on Ineffective Erythropoiesis (IE):

  Erythroferrone (ERFE); soluble transferrin receptor 1 (sTfR1); reticulocyte count; reticulocyte Hb content (CHr); erythropoietin (EPO); growth differentiation factor 15 (GDF15); bone marrow iron concentration.

- Evaluate the impact of ISIS 702843 on measures of iron overload:

  LIC; serum transferrin saturation (TSAT); serum ferritin.

- Evaluate the efficacy of ISIS 702843 by demonstrating an increase in serum hepcidin concentration.
- Evaluate the efficacy of ISIS 702843 by demonstrating improvement in anemia parameters:

  Hb; RBC count; hematocrit; MCV; mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC)

- Evaluate the impact of ISIS 702843 on hemolysis:

  bilirubin (indirect); lactate dehydrogenase (LDH)

- Evaluate the impact of ISIS 702843 on coagulation profile:

  Prothrombin time (PT); international normalized ratio (INR); activated partial thromboplastin time (aPTT)

- Evaluate the impact of ISIS 702843 on disease severity:

  Number of transfusions; chelator usage

1.2.4 Safety Objectives

- Evaluate the safety and tolerability of multiple doses of ISIS 702843 in patients with β-Thalassemia Intermedia.
- Evaluate the impact of multiple doses of ISIS 702843 on the emergence and/or progression of recognized complications of β-Thalassemia Intermedia.

1.2.5 Pharmacokinetic objectives

Evaluate pharmacokinetic (PK) exposure over time and potential PK / pharmacodynamic (PD) correlation on relevant biomarkers and efficacy outcome measures.

1.3 Endpoints

1.3.1 Primary Endpoint

Proportion of patients who have a ≥ 1.0 g/dL increase from Baseline in Hb, comparing the 3 cohorts at Week 27 of treatment.

1.3.2 Secondary Endpoints

- Proportion of patients who have a ≥ 1.5 g/dL increase from Baseline in Hb, comparing the 3 cohorts at Week 53 of treatment.
- Proportion of patients who have a ≥ 1.0 mg Fe/g dry weight decrease from Baseline in LIC, comparing the 3 cohorts at Week 53 of treatment.

1.3.3 Exploratory Endpoints

- Change from baseline of IE assessment, including plasma erythroferrone concentration, plasma sTfR1 concentration, reticulocyte count, reticulocyte Hb content (CHr), plasma EPO concentration, plasma GDF15 concentration, and bone marrow iron concentration, comparing the 3 cohorts over time.
- Change from baseline of iron overload assessment, including: LIC, TSAT, serum ferritin, chelator usage, and hepcidin, comparing the 3 cohorts over time.
- Change from baseline of anemia assessments including: Hb, RBC count, hematocrit, mean corpuscular volume (MCV), mean corpuscular Hb (MCH) amount, and corpuscular Hb concentration (MCHC), comparing the 3 cohorts over time.
- Change from baseline of hemolysis assessment, including plasma bilirubin (indirect), and plasma LDH, comparing the 3 cohorts over time.
- Change from baseline of hypercoagulability profile, including PT, INR, and aPPT, comparing the 3 cohorts over time.
- Change from baseline on disease severity, including change in the number of transfusions and chelator usage, comparing the 3 cohorts over time.

1.3.4 Safety Endpoints

- Adverse events (AEs), vital signs, physical examination findings, clinical laboratory tests (serum chemistry, hematology, urinalysis, coagulation panel, thyroid panel), electrocardiogram (ECG).
- Measures of extramedullary hematopoiesis, liver function, splenomegaly, osteoporosis, diabetes, and pulmonary hypertension (PHT).

1.3.5 Pharmacokinetic Endpoints

A PK profile (including pre-dose sampling) over a 6-hour period will be collected at Day 1 (Week 1). Two (2) samples, pre-dose and 3-hour, will be evaluated at Day 197 (Week 29). Trough levels will be evaluated just prior to each dose through Day 169 (Week 25), as well as on a more limited basis throughout the rest of the Treatment Period. One (1) sample will be evaluated on Day 183 (Week 27); there is no dose at this visit. In the Post-Treatment Period, 1 sample per each of the 3 planned visits will be evaluated, as available.

2 PROCEDURES

2.1 General Overview of Procedures

Ionis Pharmaceuticals, Inc. (or designee) will review all study data including source documents, case report forms, and laboratory reports. The study site will enter subject source data into the case report form. Some laboratory data will be transferred electronically from PPD Development (plasma PK) to Ionis Pharmaceuticals, Inc. Additional lab data will be transferred from Medpace Reference Laboratories (MRL) as the central lab, as well as from other specialty laboratories.

2.2 Randomization & Treatment Allocation

Patients were randomized after all screening assessments were completed and after the Investigator verified that they are eligible per criteria in protocol Sections 5.1 and 5.2. No subject began treatment prior to randomization and assignment of a unique subject identification number.

Eligible patients were stratified based on screening Mean Hb (≤ 8.0 g/dL or > 8.0 g/dL) and then patients were randomized. Under the original Protocol and Protocol Amendment 1, approximately 36 patients were to be randomized into 3 treatment cohorts (ISIS 702843 30 mg, 50 mg, or 80 mg) in a ratio of 1:1:1. However, upon approval of Protocol Amendment 2, patients were randomized in parallel to Cohort B (120 mg ISIS 702843 per dose) or Cohort C (120 mg ISIS 702843 per dose for 3 consecutive doses, followed by 160 mg ISIS 702843 per dose, if dose escalation is allowed based on a demonstration of adequate safety), following a prespecified randomization schema. No patients were randomized to Cohort A after Amendment

2 approval. A summary of treatment cohort and planned dose is presented in Table 1, Section 8.1 in the protocol.

Eligible patients must have a clinical diagnosis of β-Thalassemia Intermedia with genotypic confirmation of β-globin gene mutation and must be non-transfusion dependent in order to participate in the study (see protocol Section 5.1 Inclusion Criteria). The Sponsor or designee prepared the randomization list and utilized an automated IRT (Interactive Response Technology) system.

2.3 Conduct

The study was conducted in accordance with current Good Clinical Practice (GCP) and International Conference on Harmonization (ICH) guidelines, the Food and Drug Agency (FDA) Code of Federal Regulations, and all other local regulatory requirements.

2.4 Data Monitoring

2.4.1 Safety Data Monitoring

Ionis Pharmaceuticals, Inc. (or designee) is responsible for processing all reported adverse events (AEs). All serious adverse events (SAEs), reported to Ionis Pharmaceuticals, Inc. (or designee), are reviewed according to standard operating procedures. The medical monitor will review all AEs and SAEs on an ongoing basis throughout the study. Ionis Pharmaceuticals, Inc. (or designee) will prepare and submit safety reports to the health authorities worldwide in accordance with local requirements. If it becomes necessary to communicate new safety information, Ionis Pharmaceuticals, Inc. (or designee) will also prepare a safety notification letter and transmit it to all applicable study sites.

2.4.2 Data Monitoring Board

An internal safety committee was planned, as described in Section 3.6 of the protocol. However, this committee did not meet because the Sponsor Medical Monitor prepared medical monitoring reports at a frequency of approximately every 1 to 2 months, no concerning safety trends were identified, and the study was terminated early by the Sponsor due to a lack of efficacy.

2.5 Data Management

An electronic case report form (eCRF) utilizing an Electronic Data Capture (EDC) application was used for this Study.

2.5.1 Case Report Form Data

Clario (or designee) is responsible for creating the Electronic Data Capture (EDC) data entry screens, database and edit checks using definitions developed by Ionis Pharmaceuticals, Inc. Ionis Pharmaceuticals, Inc. is responsible for the review, data management querying and locking of the database.

Data are single-entered into the EDC system by the investigator site staff. Programmed edit checks (computer logic that checks the validity of the data entered and also prompts for missing data that is expected to be entered) are run and automatic queries are generated. Ionis Pharmaceuticals, Inc. reviews all data for accuracy and validity and generates additional queries in the EDC system when necessary. The data is corrected or an explanation concerning the query is provided in the EDC system. After all data are entered, source data verified, reviewed and queried, the database is closed. The data is then reviewed by Ionis Pharmaceuticals, Inc. and additional queries may be generated. After all queries are resolved, the database is locked.

2.5.2 Laboratory Data

Ionis Pharmaceuticals, Inc. is responsible for the format of the laboratory electronic data transfers, transfer schedule and review of the clinical laboratory data. For the central lab, the data is not stored in the EDC system. Investigator sites have access to the data via lab reports sent directly from the laboratory and online using the MRL ClinTrak® database. In addition, Investigators may, at their discretion, test specific laboratory parameters at their local laboratory for safety assessments prior to dosing. In these instances, the local laboratory results will be recorded by the site into the EDC system. The final lab data will be stored as SAS data sets.

For specialty labs such as Blueprint Genetics, BioPharma Diagnostics (Mayo), Medpace Core Laboratories, QPS Holdings, L.L.C., Radboud, Resonance Health, Ionis Research Lab, and Versiti Blood Center of Wisconsin, data will be transferred directly to Ionis per each vendor's Data Transfer Agreement, if applicable.

2.5.3 Pharmacokinetics Data

Ionis Pharmaceuticals, Inc. is responsible for the management and review of the plasma drug concentration data from PPD®. This process involves reviewing the subject and visit identifiers (i.e., subject demographics) with the clinical data collected in the EDC system. The PK data will be stored as CSV and/or EXCEL files. Final data, which has been approved by Quality Assurance, will be stored in the Sponsor's document management system.

2.5.4 Immunogenicity (IM) Analysis

Ionis Pharmaceuticals, Inc. is responsible for the management and review of the immunogenicity sample data from Charles River Laboratories-Montreal. This process involves reviewing the subject and visit identifiers (i.e., subject demographics) with the clinical data collected in the EDC system. The ADA data will be stored as CSV and/or EXCEL files. Final data, which has been approved by Quality Assurance, will be stored in the Sponsor's document management system.

3 ANALYTICAL PLAN

3.1 Statistical Design Summary

The study enrolled 29 patients overall, with 12 patients in Cohorts A and B combined, and 17 patients in Cohort C . The eligible patients were stratified based on a screening mean Hb level status [low (≤ 8.0 g/dL) or high (> 8.0 g/dL)]. There is no statistical rationale for the selected sample size. Each patient was to be treated for up to 2 years. The outcomes are descriptive.

Two (2) interim analyses were planned. Refer to Section 3.3.4 for the timing and the objectives of the interim analyses. However, neither interim analysis was formally performed (i.e., with a database lock) because it became clear from safety monitoring that blood hemoglobin was not increased before the planned analyses were to be conducted. In lieu of a formal first interim analysis, an analysis of the available data was conducted, and the protocol was amended to allow higher doses to be tested. In lieu of a formal second interim analysis, the available data were analyzed, and after careful consideration the decision was made to terminate the study early.

3.2 General Overview of Analyses

3.2.1 Statistical Methods

Descriptive summary statistics including number of patients, mean, median, standard deviation, standard error of mean, 25th percentile, 75th percentile, minimum, and maximum for continuous variables, and counts and percentages for categorical variables will be provided to summarize most data. Additional subject listings included case report form (CRF) data and derived outcomes from the data may be presented.

Unless otherwise specified, the study outcomes will be summarized by treatment cohort (Cohort A, Cohort B, Cohort C, Cohorts A and B combined, and total).

For the categorical outcomes, the data will be analyzed by using one-sample Exact test to evaluate whether the estimate proportions are deviated from zero. For the continuous outcomes, the data will be analyzed using one-sample t-test to evaluate if the changes from baseline are significantly different than zero. The normality assumption for one-sample t-test model will be assessed by the Shapiro-Wilk test. In the case of data departs substantially from normality, the nonparametric Wilcoxon signed rank test will be applied instead.

All statistical tests will be conducted using 2-sided tests with 5% Type I error rate unless otherwise stated. Where appropriate, p-values will also be provided for statistical tests.

For Hb, the collected Hb values within 6 weeks after the time of receiving an RBC transfusion will be considered as missing in the efficacy analyses. Mean, mean of absolute change from baseline, and mean percent change from baseline over time by treatment cohort will be plotted

for the selected analytes in the efficacy analyses. Given the limited size of the cohort, median of the summary over time by treatment cohort plot may also be provided.

Baseline Definition

The baseline for Hb and other hematology parameters is defined as the average of Screening period and Day 1 pre-dose measurement. Each measurement used for the baseline calculation must be taken at least 6 weeks away after the most recent transfusion. Baseline must be based on at least two measurements.

The baseline for all other assessments is defined as the last non-missing measurement prior to the first dose of study drug. For ECG, the baseline is defined as the average of the last non-missing triplicate prior to the first dose of study drug.

Analytical Visits

In general, all post-baseline data will be summarized using the visit labels provided in the data. Multiple results with the same visit label will be averaged for the continuous variables, and the worst result will be used for the categorical variables. Results with visit labels as "Unscheduled" will not be included in the by-visit summary tables and figures except for determining baseline and the incidence of abnormality lab summary including AST, ALT, and platelet number specified in Section 3.7.3 but will be presented in data listings. Other situations, such as required additional confirmation, will be described separately in the related endpoint analysis approach.

Disposition of Subjects

The number of patients screened, the number of patients screen failures, the number and percentage of patients enrolled, the number and percentage of patients completed the study treatment, the number and percentage of patients who discontinued from treatment early and reason, the number and percentage of patients completed post-treatment follow-up, and who discontinued from post-treatment follow-up and reason will be summarized. A by-patient listing will also be provided.

Additional summaries will be provided to summarize the number of patients with screen failure, and treatment/post-treatment follow-up termination incidences related to the COVID-19 public health emergency. A listing will also be provided to present the incidences above. Please refer to Section COVID-19 Related Impact for more information about this listing.

Demographic and Baseline Characteristics

Demographic, stratification factors and Baseline characteristics (e.g., age, gender, ethnicity, race, weight, height, BMI, splenectomy status, screening Hb, prior packaged RBC transfusion rate (unit/yr), and on chelation medication at Day 1) will be summarized using descriptive statistics by treatment cohort. Other disease characteristics related assessments, such as ferritin, TSAT, and LIC, will be summarized in the summary tables separately.

As for the information of race, if multiple races are selected in the database, then 'Multiple Race' is used in the summary table and the data point will only be counted once in the category of 'Multiple Race'. The listing will display the specific race information.

BMI will be computed using the formula: BMI = (weight in kilograms) / [screening height in cm / 100]^2

All protocol deviations will be listed and be summarized. An additional summary table by deviation type and classification will be provided to illustrate the protocol deviations that are related to the COVID-19 public health emergency.

The protocol deviations related to the COVID-19 public health emergency will be listed separately. Please refer to Section COVID-19 Related Impact for more information about this listing.

COVID-19 Related Impact

A listing of all patients affected by the COVID-19 public health emergency related study disruption will be provided. The listing will include the information in the following category with corresponding results and/or comments, if available.

- Screen failure
- Treatment termination/ Follow-up termination
- Protocol deviation
- Local lab data

3.2.2 Subject Population Analyzed

The following analysis populations are defined for this study:

- Safety Set: All randomized patients who received at least 1 dose of ISIS 702843.
- PK Set: All randomized patients who received at least 1 dose of ISIS 702843 and have at least 1 evaluable PK sample.
- Full Analysis Set (FAS): All randomized patients who received at least 1 dose of ISIS 702843 and who have at least 1 post-baseline Hb assessment after Day 1.

ln addition to the above analysis sets, it is recognized that some data displays will be provided for "All Screened", "Screening Failures", and "All Randomized" subjects but no data analysis is planned to be executed in these populations.

3.3 Primary Analysis

3.3.1 Primary Endpoint Definition

The proportion of patients who have a ≥ 1.0 g/dL increase from Baseline in Hb at Week 27 of treatment in the FAS.

3.3.2 Analysis of Primary Endpoint

The primary analysis will be the proportion of patients achieving ≥ 1.0 g/dL increase from baseline at Day 183 (Week 27) in Hb summarized by treatment cohort in the FAS. The data will be analyzed using one-sample Exact test as described in Section 3.2.1. Ninety-five (95%) exact confidence interval of the response rate will also be provided.

The value to use for Week 27 result is the mean of the 2 Hb measurements intended to be taken at Day 169 (Week 25) and Day 183 (Week 27); if one of these measurements is missing, then the other measurement will be used for Week 27 result. For a Hb measurement to be usable for Week 27 result, the sampling must occur at least 6 weeks after the most recent transfusion for that patient. The end time of the transfusion will be derived as 4 hours from the start time.

Based on preliminary analysis (see Section 3.3.4), only comparison between week 27 and baseline within each cohort will be provided.

3.3.3 Sample Size Consideration

The sample size was based on prior experience with second-generation ASOs to ensure that the safety, tolerability, efficacy, and PK of ISIS 702843 will be adequately assessed while minimizing unnecessary subject exposure. The maximum enrollment of patients was expected to be 30 (or until enrollment is closed by the Sponsor). The Sponsor closed enrollment to Cohorts A and B before enrollment to Cohort C because it was considered sufficient to have approximately 12 patients in Cohorts A and B combined.

For a set of 12 enrolled patients, the chance to observe no responder in primary endpoint is around 28% if the true response rate is 10%, but the chance to observe no responder decreases to around 1% if the true response rate becomes 30%.

3.3.4 Planned Interim Analyses

Two (2) interim analyses (IAs) were planned:

- When at least 20 patients had completed Day 183 (Week 27) assessments. The main purpose of this IA was to evaluate ISIS 702843 efficacy effect on Hb, other hematology measures, and Iron panel measures. The information was to be used to support decisions related to the potential for dose adjustments applicable to an entire cohort.

- When at least 16 patients had completed Day 365 (Week 53) assessments. The main purpose of this IA was to evaluate ISIS 702843 efficacy effect on LIC and further on the measures associated with the first interim analysis.

However, neither IA was formally performed (i.e., with a database lock) because it became clear from safety monitoring that blood hemoglobin was not increased before the planned analyses were to be conducted. In lieu of a formal first interim analysis, the available data were analyzed, and the protocol was amended to allow higher doses to be tested. In lieu of a formal second interim analysis, the available data were analyzed, and the decision was made to terminate the study.

3.3.5 Incomplete or Missing Data

Missing values will not be imputed. Patients with missing data for a scheduled assessment time point were excluded from the summary for that time point.

3.4 Secondary Analyses

3.4.1 Secondary Endpoint Definitions

- Proportion of patients who have a ≥ 1.5 g/dL increase from Baseline in Hb at Week 53 of treatment.
- Proportion of patients who have a ≥ 1.0 mg Fe/g dry weight decrease from Baseline in LIC at Week 53 of treatment.

3.4.2 Analysis of Secondary Endpoints

The secondary efficacy analyses will be performed in a similar way to the primary efficacy analysis and conducted using the FAS. The proportion of patients achieving ≥ 1.5 g/dL increase from baseline at Day 365 (Week 53) in Hb summarized by treatment cohort in the FAS. For a Hb measurement to be usable for the determination of the value to use for Week 53 of treatment, the sampling must occur at least 6 weeks after the most recent transfusion for that patient. The value to use for Week 53 result is the mean of the 2 Hb measurements intended to be taken at Day 309 (Week 45) and Day 365 (Week 53); if one of these measurements is missing, then the other measurement will be used for Week 53 result. For a Hb measurement to be usable for Week 53 result, the sampling must occur at least 6 weeks after the most recent transfusion for that patient.

The proportion of patients who have a ≥ 1.0 mg Fe/g dry weight decrease from Baseline in LIC at Week 53 will be summarized by treatment cohort in FAS. The Week 53 value is the LIC measurement intended to be taken at Day 365 (Week 53).

Based on preliminary analysis (see Section 3.3.4), only comparison between Week 53 and baseline within each cohort will be provided.

3.5 Exploratory Analyses

The exploratory endpoints listed in Section 1.3.3 will be summarized by treatment cohort in the FAS. The analyses based on the outcome characteristics (continuous or categorical) will be summarized by scheduled visits. For continuous outcomes, mean, mean of absolute change from baseline, and mean percent change from baseline over time will be summarized by scheduled visits, and treatment cohort.

The number and percentage of patients who achieved additional response criteria for key iron parameters will be tabulated also. For Hb, the response criteria will include increase by ≥ 1.0 g/dL from baseline; increase by ≥ 1.5 g/dL from baseline. For LIC, the response criteria will include reduction by 1.0 mg Fe/g dry weight from baseline. For TSAT, the response criteria will include reduction by ≥ 10% from baseline; reduction by ≥ 20% from baseline; reduction by ≥ 30% from baseline; reduction by ≥ 50% from baseline. Percentages are calculated based on the number of patients with available samples at certain visit.

Individual plots with several selected analytes over time may be provided.

Based on preliminary analysis (see Section 3.3.4), only comparison between week 53 and baseline for Hepcidin within each cohort will be provided.

The following exploratory endpoints as described in the protocol will not be analyzed since the Sponsor terminated the study due to a lack of efficacy. The study team has decided not to present this part of analyses in the abbreviated CSR because primary and secondary endpoints were not met:

- Change from baseline of erythroblast count
- Change from baseline of spleen iron concentration, and non-transferrin bound iron (NTBI)
- Change from baseline of plasma haptoglobin, plasma protoporphyrin IX, and blood smears
- Change from baseline of antithrombin III, Protein S, and Protein C
- Change from baseline on quality of life measures, including change from baseline in NTDT-PRO©, SF-36, PGIC, ISGA, and FACIT-Fatigue
- Change from baseline on functional status of patient using 6MWT instrument

3.6 Pharmacokinetic (PK) and Immunogenicity (IM) Analysis

Pharmacokinetic (PK) analysis will be conducted in the PK Population. The plasma pharmacokinetics of ISIS 702843 (as total full-length antisense oligonucleotide (ASO) or

ISIS 702843-equivalent, ISIS 702843-eq., including fully conjugated, partially conjugated, and unconjugated ISIS 702843) will be assessed following SC dose administration.

3.6.1 Plasma Concentration Data of Total Full-Length Oligonucleotides

Plasma concentrations of ISIS 702843 (ISIS 702843eq.), along with the scheduled (nominal) and actual sample times (i.e., time from SC dosing) will be listed (when applicable) for each patient, by dose cohort, nominal and actual dose, study day, scheduled and actual time point, and dose number with respect to nominal dose assigned by relevant protocol amendment. In addition, percent differences between scheduled and actual sampling times will be listed for all patients, as well as percent differences between actual administered dose and nominal dose.

Plasma concentrations below the lower limit of quantification (LLOQ) will be indicated by "BLQ". For the purpose of calculating typical descriptive statistics (n, mean, SD, %CV, geometric mean, geometric %CV, median, minimum, and maximum) for plasma concentrations, all BLQ values will be set to zero. Mean plasma concentrations that are BLQ will be presented as "BLQ", and the SD and %CV, geometric mean and geometric % CV will be reported as not applicable. Summary statistics of the ISIS 702843 plasma concentrations will be tabulated by nominal dose as assigned per relevant protocol amendment, dose number with respect to nominal dose assigned at randomization and subsequently per relevant protocol amendment, nominal study day, and scheduled time point. At the discretion of the pharmacokineticist and/or biostatistician, samples may be excluded from descriptive statistics if there are large deviations between scheduled and actual sampling times, or large deviations between actual dose and nominal dose.

ISIS 702843eq. plasma concentration versus time (actual) profiles for each patient that received ISIS 702843, as well as the mean (± SD or SE) plasma concentrations versus time (scheduled) profiles, will be presented graphically on linear and semilogarithmic scales. At the discretion of the pharmacokineticist and/or biostatistician, samples may be excluded from the mean plots if there are large deviations between scheduled and actual sampling times.

3.6.2 Plasma Pharmacokinetic Parameters

The plasma PK of ISIS 702843 (as total full length ASO) will be assessed following the first SC dose, as data allows. Non-compartmental PK analysis of ISIS 702843 (total full-length ASO) will be carried out on each individual subject data set using Phoenix WinNonlin version 8.0 or higher (Pharsight Corporation, Mountain View, CA). For calculation of PK parameters, all BLQ values will be set to zero. The plasma PK parameters for ISIS 702843eq. will be calculated based on actual sampling times. The plasma PK parameters to be calculated or determined (when applicable) are listed in Table 2. Other plasma PK parameters, as appropriate, may be determined or calculated at the discretion of the pharmacokineticist.

Table 2 Plasma Pharmacokinetic Parameters to be Calculated or Determined

Parameter	Definition/Method	Study Day 1
C_{max}	Maximum observed concentration	X
T_{max}	Observed time at which C_{max} occurs	X
T_{last}	Time of last measurable (positive) concentration	X
AUC_{0-t}	Partial AUC: Area under the concentration-time curve from time zero to time t (e.g., 6 hr, as applicable), calculated using linear-up log-down method	X
CL_{0-t}/F	Partial clearance (e.g., 6 hr) divided by F (fraction of the dose absorbed) determined by Dose/AUC_{0-t}	X
$t_{1/2\lambda z}$	For all evaluable patients who have sufficient post-treatment samples collected, apparent terminal elimination half-life will be calculated from the equation, $t1/2\lambda z = 0.693/\lambda z$, where λz is the rate constant associated with the apparent terminal elimination phase. A minimum of three data points in the elimination phase will be used to define λz, a span of at least 1.5, and the correlation of determination values (r^2 adjusted) must be at or greater than 0.8 for the estimate to be accepted. This parameter will only be calculated following the final dose on Day 729 for all evaluable patients receiving active study drug.	X

Note: X designates parameters to be calculated or determined assuming sufficient data

Plasma pharmacokinetic parameters will be summarized using descriptive statistics (n, mean, SD, %CV, geometric mean, geometric %CV, median, minimum, and maximum) by nominal dose and study day. Summary may also include dose number with respect to nominal dose assigned at randomization and subsequently per relevant protocol amendment.

3.6.3 Pharmacokinetic/Pharmacodynamic Exposure-Response Analysis

Exposure-response correlations may be explored graphically between plasma exposure (i.e., AUC, C_{max}, C_{trough}, as appropriate), and selected PD measures (e.g., hemoglobin), and/or other relevant biomarkers (such as hepcidin, TSAT, serum iron, etc.).

Population PK and PK/PD analysis may be performed using the PK and PD data from this Study, and/or combined with other ISIS 702843 clinical PK/PD data from any previous and future studies in the development timeline.

3.6.4 IM Analysis

Anti-drug antibody (ADA) analyses will be conducted in the 'Safety Set'. Samples collected for ADA assessment at selected time points will be analyzed for anti-ISIS 702843 antibodies (i.e., anti-drug antibodies; ADA).

3.6.4.1 Sample Level ADA data

An evaluable sample will be designated 'IM positive' based on both positive screening and confirmation assay results (i.e., confirmed positive result), and otherwise will be deemed 'IM negative'. Sample IM results (screen positive/negative, confirmed positive/negative or unevaluable, and when applicable, titer of anti-ISIS 702843 antibodies) before, during, and after treatment with ISIS 702843 (sample ADA status) will be listed for each subject by treatment, dose (nominal and actual), dose number with respect to nominal dose assigned at randomization and subsequently per relevant protocol amendment, as well as study day of collection (nominal and actual).

The sample ADA incidence (number) and incidence rate (percent) at each evaluated study time point will be determined and appropriately summarized by treatment and dose as the total number of and percentage of evaluated subjects with sample ADA negative, positive, and unknown status. Furthermore, titer over time will be also appropriately summarized (using descriptive statistics) as median, quartiles (25% and 75%), and range by treatment and dose. Summary may also include dose number with respect to nominal dose assigned at randomization and subsequently per relevant protocol amendment.

3.6.4.2 Subject Level ADA data

Subject ADA status overall (ADASTAT) will be defined as 'Positive' if they have at least 1 confirmed positive sample result at any time during the treatment or post-treatment evaluation periods; 'Negative' if all evaluated ADA sample results during the treatment and post-treatment evaluation periods are ADA negative and they have at least one evaluable ADA result collected post study drug treatment. Otherwise, a study patient will be assigned 'Unknown' ADA status.

Furthermore, subjects with positive overall ADA status will be further classified into different ADA types based on their baseline ADA status and change in ADA titer post treatment as described below (Shankar et al. 2014):

- Treatment-Emergent ADA: sum of treatment-induced ADA and treatment-boosted ADA as described below:
    - Treatment-Induced ADA: ADA developed de novo (seroconversion) following biologic drug administration (i.e., formation of ADA any time after the initial drug administration in a subject without pre-existing ADA, i.e., baseline negative ADA)
    - Treatment-Boosted ADA: pre-existing ADA that were boosted to a higher level following biologic drug administration (i.e., any time after the initial drug administration the ADA titer is greater than the baseline titer by a factor of 8-fold or more)

- Treatment-Unaffected ADA: pre-existing ADA that were not affected (boosted) following biologic drug administration (i.e., any time after the initial drug administration the ADA titer is 4-fold or less)
- ADA type would be not applicable (NA) if the subject overall ADA status is negative.

Other subject level IM parameters to be calculated/defined may include but are not limited to:

- Subject ADA Status at Baseline (ADASTATB): "Positive" if the subject has Week 1 Day 1 pre-dose sample (baseline) tested as confirmed positive; "Negative" if the subject has Week 1 Day 1 pre-dose sample (baseline) tested as confirmed negative; "Unknown" if the subject has Week 1 Day 1 pre-dose sample (baseline) that is unevaluable.
- Onset of ADA (TFSTADA): i.e., the first day ADA positive sample observed, will be calculated by: the date first sample has “positive” IM status - first dose date +1. This parameter will be calculated for subjects with positive ADA status overall and subjects with treatment-induced ADA, respectively.
- Last Positive ADA Study Day (TLSTADA): defined as the last positive ADA sample observed from the start of study drug treatment and will be calculated by: the date of last sample “positive” IM status - first dose date +1.
- Last IM Sampling Study Day (TLSTSAMP): defined as the last ADA sample collected from the start of study drug treatment and will be calculated by: the date of last sample collected - first dose date +1.
- Peak titer (PEAKTIT): the highest titer observed for the subject
- Time to peak titer (TPEAKTIT): the time to reach peak titer will be calculated by: the date of first peak titer observed- first dose date +1
- Total number of ADA Positive Samples (NOPOSAMP): the total number of ADA samples being confirmed positive for the subject
- Total number of ADA Samples evaluated (NOADASAMT): the total number of ADA samples being collected and analyzed successfully with reportable results for the subject
- Percentage of Positive Samples (PCPOSAMP): the percentage of ADA samples being confirmed positive for the subject and will be calculated by:

$$\text{PCPOSAMP (\%)} = 100 \times \frac{\text{NOPOSAMP}}{\text{NOADASAMT}}$$

Lastly, subjects with positive ADA status may further be classified as having transient or persistent ADA response, if there are a sufficient number of subjects with transient ADA status. Transient and persistent ADA definitions are defined below and based on Shankar 2014 (Shankar et al. 2014):

Transient ADA response:

- Treatment-induced ADA detected only at one sampling time point during the treatment or follow-up observation period (excluding the last sampling time point, which will be considered persistent unless shown to be undetectable at a later time) or
- Treatment-induced ADA detected at two or more sampling time points during the treatment (including follow-up period if any), where the first and last ADA-positive samples (irrespective of any negative samples in between) are separated by a period less than 16 weeks, and the subject's last sampling time point is ADA-negative.

Persistent ADA response:

- Treatment-induced ADA detected at two or more sampling time points during the treatment (including follow-up period if any), where the first and last ADA-positive samples (irrespective of any negative samples in between) are separated by a period of 16 weeks or longer or
- Treatment-induced ADA detected only at the last sampling time point of the study treatment period or at a sampling time point with less than 16 weeks before an ADA-negative last sample.

The subject level ADA prevalence, incidence, and positive ADA response being transient or persistent (if applicable) will be calculated as the number and the proportion (percent) of the study population during the study period by treatment and dose. Subject level IM parameters (as described above) will be listed by treatment and dose for all evaluable subjects, and also appropriately summarized (using descriptive statistics) as median, quartiles (25% and 75%) and range, by treatment and dose. Additionally, Subject level IM parameters will be summarized for subjects with Treatment-Induced ADA only (i.e., excluding subjects with pre-existing ADA). Summary may also include dose number with respect to nominal dose assigned at randomization and subsequently per relevant protocol amendment.

3.6.4.3 Evaluation of IM Impact on PK, PD, Efficacy and Safety

The impact of IM on PK, PD and safety may be evaluated by stratifying plasma PK parameters, plasma trough and post-treatment ISIS 702843 concentrations and PD biomarker levels, selected clinical efficacy endpoints and safety measures by subject ADA status and titer quartiles, summarized using typical descriptive statistics, and presented graphically and/or in tables. The impact of IM may also be evaluated on selected clinical efficacy end points and safety measures.

Additionally, within subject comparisons on plasma Cmax and AUC may be conducted in patients from the PK subgroup and presented graphically by subject IM status and treatment if deemed appropriate (Wang et al. 2016).

Lastly, other stratifications (e.g., based on antibody titer, onset of ADA, etc.) of selected PK, efficacy, and safety assessments may also be performed if deemed warranted at the discretion of the pharmacokineticist, medical monitor, and/or biostatistician.

3.7 Safety Analyses

The safety analysis will be conducted on the Safety Set.

The study team has decided not to present the following safety endpoints analyses in the abbreviated CSR:

- Echocardiogram and DEXA
- Ultrasound and MRI assessment for spleen, and bone marrow
- Leg Ulcers

3.7.1 Adverse Events

The incidence of AEs will be summarized by Medical Dictionary for Regulatory Activities (MedDRA) version 22.0 (or higher) preferred term and system organ class for:

- Any treatment-emergent adverse events (TEAEs)
- Related TEAEs. Related is defined as “Related”, “Possible”, or missing relationship to study drug
- Any TEAEs by severity. At each level of subject summarization, a subject is classified according to the highest severity if the subject reported one or more events. Adverse events with missing severity will categorized as “Missing” for this summary
- Serious TEAEs (SAEs)
- TEAEs leading to permanent study drug discontinuation

SAEs and non-serious AEs that lead to study discontinuation or study drug discontinuation will be listed separately. All AEs, including SAEs, will be presented in a subject listing, and non-treatment-emergent adverse event will be noted in the listing.

To determine the AE as treatment-emergent or not, if there is no “Formlink” link, and the AE (start date/time) occurs after the subject’s first dosing date/time, then the AE is “treatment-emergent”. Otherwise, if the AE (start date/time) occurs prior to the subject’s first dosing date/time, then the AE is not treatment-emergent.

In addition, if there is a "Formlink" link between two AE records, the 2 records will be analyzed pairwise. Consider the following 2 cases, where the AE severity (mild/moderate/severe) are compared between the 2 records in the pair. The 2 records will be ordered chronologically (by AE start date) and referred to the "first" and "second" AE.

Case 1: The first AE record in the pair occurs before first dosing, and the second record occurs after first dosing.

If the AE severity of the second record is worse than that of the first record, then only the second AE is deemed a TEAE. Otherwise, neither record is considered as TEAE.

Case 2: Both AE records in the pair occur after first dosing.

The worst AE record will be deemed a TEAE.

All TEAEs identified based on the rules above will be summarized in the event number analysis.

The most conservative approach will be used to determine if the event occurs after the treatment. For example, if the onset date or resolution date of an AE is prior to the first study treatment date, it will be considered to have occurred prior to the study period. If the onset or resolution date of an AE is a partial date with only month or year available or completely missing, then the event is assumed to be within the study period unless the year is prior to the year of the first study treatment date, or if in the same year, the month is prior to the month of the first study treatment date.

Handling of Missing Data

If the date of adverse events or prior/concomitant medications is completely or partially missing, the data will be imputed by the rules below.

For AEs, the following imputation rules will be applied to impute start dates under conservative principles:

- If year, month and day are all missing then assign the date of first dose of Study Drug
- If month and day are missing and year is:
  - the same as the year of the first dose of Study Drug then assign the month-day of first Study Drug
  - earlier than the year of the first dose of Study Drug then assign December 31
  - after the year of the first dose of Study Drug then assign January 1
- If only day is missing and month-year is:
  - the same as the month-year of the first dose of Study Drug then assign the day of first Study Drug
  - earlier than the month-year of the first dose of Study Drug then assign the last day of the month

  - after the month-year of the first dose of Study Drug then assign the first day of the month

Imputation will be performed only for the end date only if the day or month is missing (i.e., year is present) for a resolved AE as follows:

- If month and day are missing and year is
  - the same as the year of the last dose of Study Drug then assign the month-day of the last dose of Study Drug
  - otherwise, assign December 31
- If only day is missing then assign the last day of the month

If the imputed start date is later than the imputed end date then set the imputed start date to the imputed end date.

For prior/concomitant medications, the following imputation rules will be applied to impute start dates under conservative principles:

- If year, month and day are all missing then assign the date of first dose of Study Drug
- If month and day are missing and year is:
  - earlier than the year of the first dose of Study Drug then assign December 31
  - otherwise, assign January 1
- If only day is missing and month-year is:
  - earlier than the month-year of the first dose of Study Drug then assign the first day of the month
  - otherwise, assign the last day of the month

Imputation will be performed only for the end date only if the day or month is missing (i.e., year is present) for a stopped prior/concomitant medication as follows:

- If month and day are missing then assign December 31
- If only day is missing then assign the last day of the month

If the imputed start date is later than the imputed end date then set the imputed start date to the imputed end date.

Local Cutaneous Reactions at the Injection Site

Local cutaneous reactions at the injection site (LCRIS) is defined as (A) moderate or severe adverse events with the preferred terms (PTs) including Injection site erythema, Injection site swelling, Injection site pruritus, or Injection site pain that started on the day of injection, persisted for at least 2 days or ongoing; or (B) any AE at the Study Drug injection site, regardless of severity, that leads to discontinuation of study drug, where AE at the Study Drug injection site is the principal reason for discontinuation.

Percentage of injections leading to LCRIS will be calculated as follows for each subject: (A/B)*100, where A = number of injections with an LCRIS, and B = total number of injections. Doses that are split across multiple injections are counted as a single injection.

LCRIS will be summarized using the MedDRA coding system, by System Organ Classes (SOCs) and PTs. Percentage of injections leading to LCRIS will also be summarized.

LCRIS will be listed.

Flu-like Reactions

Flu-like reactions (FLR) will also be summarized using the MedDRA coding system, by SOCs and PTs.

Flu-like reactions are defined as adverse events with PTs including either (A) Influenza like illness or (B) Pyrexia or Feeling hot or Body temperature increased, plus at least 2 of the following symptoms with the PTs: Chills, Myalgia, or Arthralgia starting on the day of injection or the next day.

Percentage of injections leading to flu-like reactions will be summarized using the descriptive statistics and calculated as follows for each subject: (A/B)*100, where A = number of injections leading to flu-like reactions, and B = total number of injections. Note that doses that are split across multiple injections are counted as a single injection.

Percentage of injections leading to FLRs will also be summarized.

FLRs will be listed.

AE of special interest (AESI)

There is no AESI for this study.

3.7.2 Vital Signs Measurements

Vital signs will include weight, respiratory rate, body temperature, BMI, and systolic and diastolic blood pressure. All vital signs will be summarized by treatment cohort for vital sign values as well as the change and percent change from baseline at each post-baseline visit.

3.7.3 Laboratory Measurements

Chemistry, hematology, coagulation, complement, and urinalysis (result, mean change, and mean percent change from baseline) will be summarized by treatment cohort and each post-baseline visit. For urinalysis, only Protein, Creatinine, and protein-to-creatinine (P/C) ratio will be summarized. Listing of laboratory assessments in Chemistry, Hematology, and urinalysis will be provided. Local laboratory data will also be provided in the listings separately.

For ALT and AST, the number and percent of patients in each of the following categories will be tabulated by treatment cohort:

- ALT/AST > 3 × ULN, confirmed
- ALT/AST > 5 × ULN, confirmed

A confirmed value is based on a consecutive lab value performed on a different day to, but within 7 days of, the initial value. If the repeated value is in the same or worse category, then the initial value is considered confirmed. If the consecutive value is in a better category, then the initial value is confirmed using the consecutive value category. If values that are not confirmed due to failure to retest or missing lab values, then the initial value is presumed confirmed.

If there are multiple results on the same day (no matter from the same lab vendor or different lab vendors), then the worst value will be utilized in the analysis.

The number and percent of subjects in the following confirmed nadir platelet counts categories will be summarized by treatment cohort:

- 100,000 to < 120,000/mm^3
- 75,000 to < 100,000/mm^3
- 50,000 to < 75,000/mm^3
- 25,000 to < 50,000/mm^3
- < 25,000/mm^3

Subjects with more than one confirmed value will be counted exactly once under the worst confirmed category.

3.7.4 Exposure and Drug Administration

Treatment duration and amount of Study Drug received will be summarized by treatment cohort. The treatment duration for each subject is defined as last treatment dose date – first treatment dose date + 1.

The time on study will be defined as the total number of days a subject is known to be followed on study calculated as follows:

Time on study = Last date on study – Date of first dose + 1,

where the last date on study is defined as the date of the latest visit with evaluation for a given subject. Visits with refused or unable to contact are not visits with evaluation.

The duration of exposure to ISIS 702843 will be summarized at each dose level for patients receiving ≥ 1 dose, ≥ 7 doses, and ≥ 13 doses every 4 weeks without interruption, to capture exposure to ISIS 702843 ≥ 1 month, ≥ 6 months, and ≥ 12 months, respectively.

The data of drug administration will be presented in listings, including dosing information, dose pause, etc.

3.7.5 12-Lead Electrocardiograms (ECG)

Safety 12 lead ECG will be performed in triplicate at the visits indicated in the protocol Appendix A, Schedule of Activities.

The ECG data will include ventricular rate (VR), PR interval, QRS duration, QT, and corrected QT intervals, and overall interpretation.

For the continuous variables above, descriptive statistics (n, mean, standard error, standard deviation, median, 25th percentile, 75th percentile, minimum and maximum) of the average of triplicate results at each study visit, as well as the change and percent change from Baseline to each study visit, will be summarized by treatment cohort ; for the categorical responses to overall interpretation, the worst of triplicate results and the associated findings at each visit will be summarized by counts and percentages. All the ECG data collected in triplicate will be listed.

3.7.6 Prior and Concomitant Medications

Prior and concomitant medications will be coded using WHO Drug dictionary (version March 2019 or later) and summarized by Anatomical Therapeutic Chemical (ATC) class, preferred terms and, by treatment cohort. Ancillary procedures, if available, will also be summarized.

As for the usage of chelation, in addition to the analyses specified in Section 3.5, the data will be summarized in the same way as prior and concomitant medications.

3.7.7 MRI

The MRI assessments for liver will be summarized at scheduled visits by treatment cohort. The data of LIC will be included in the MRI assessments.

Listings of the MRI assessments will also be provided.

3.7.8 Genetic Sample

The data of genetic sample will be presented in a by-patient listing.

4 REFERENCES

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REVISION SUMMARY

This document is made to accommodate the newly developed SAP template. Please refer to Section REVISION HISTORY for the detailed revision history.

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