DISCLOSURE

REDACTED PROTOCOL AMENDMENT 2

ACE-536-B-THAL-001

A Phase 3, double-blind, randomized, placebo-controlled, multicenter study to determine the efficacy and safety of luspatercept (ACE-536) versus placebo in adults who require regular red blood cell transfusions due to beta (β)-thalassemia.
The "BELIEVE" Trial

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A PHASE 3, DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED, MULTICENTER STUDY TO DETERMINE THE EFFICACY AND SAFETY OF LUSPATERCEPT (ACE-536) VERSUS PLACEBO IN ADULTS WHO REQUIRE REGULAR RED BLOOD CELL TRANSFUSIONS DUE TO BETA (β)-THALASSEMIA

The "BELIEVE" Trial

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PROTOCOL SUMMARY

Study Title

A Phase 3, double-blind, randomized, placebo-controlled, multicenter study to determine the efficacy and safety of luspatercept (ACE-536) versus placebo in adults who require regular red blood cell transfusions due to beta (β)-thalassemia.

Indication

Adults who require regular red blood cell (RBC) transfusions due to β-thalassemia.

Objectives

The primary objective is:

- To determine the proportion of subjects treated with luspatercept plus best supportive care (BSC) versus placebo plus BSC who achieve an erythroid response, defined as ≥ 33% reduction from baseline in transfusion burden (units RBCs / time) with a reduction of at least 2 units, from Week 13 to Week 24

The key secondary objectives are:

- To evaluate the proportion of subjects who achieve ≥ 33% reduction from baseline in transfusion burden from Week 37 to Week 48 versus placebo
- To evaluate the proportion of subjects who achieve ≥ 50% reduction from baseline in transfusion burden from Week 13 to Week 24 versus placebo
- To evaluate the proportion of subjects who achieve ≥ 50% reduction from baseline in transfusion burden from Week 37 to Week 48 versus placebo
- To evaluate the mean change from baseline in transfusion burden from Week 13 to Week 24

  Full list with Objectives and Endpoints is provided in Section 2.

Study Design

This is a Phase 3, double-blind, randomized, placebo-controlled, multicenter study to determine the efficacy and safety of luspatercept (ACE-536) plus BSC versus placebo plus BSC in adults who require regular red blood cell transfusions due to β-thalassemia. The study is divided into the Screening/Run-in Period, double-blind Treatment Period, double-blind Long-term Treatment Period, Open-label Phase and Post-treatment Follow-up Period.

The study design is described in details in Section 3.

The study will be conducted in compliance with International Conference on Harmonisation (ICH) Good Clinical Practices (GCPs).

Study Population

Subjects diagnosed with β-thalassemia (including Hemoglobin E/β-thalassemia), age ≥ 18 years, who are regularly transfused, defined as regular transfusions of 6-20 RBC units in the 24 weeks prior to randomization with no transfusion-free period > 35 days during that period.

A total of approximately 300 eligible subjects will be enrolled.

Length of Study

Study participation for each subject includes a Screening/Run-in Period of at least 12 weeks, a 48-week, double-blind, placebo-controlled Treatment Period, and if applicable, followed by a double-blind Long-term Treatment Period and an Open-label Phase. The Post-treatment Follow-up Period will last 156 weeks (3 years) following the last dose of IP (further details refer to Section 3.2).

End of Treatment for each individual subject is defined as the date of the last visit in the Treatment Period, the Long-term Treatment Period, or in the Open-label Phase, whichever is the later date.

End of Study for each individual subject occurs at the time of completion of the 156 weeks of the Post-treatment Follow-Up Period or at the time of the end of the Open-label Phase (if subjects are still on treatment, as described in Section 3.2) or at the time of the End of Trial as defined below.

The End of Trial is defined as when all subjects initially assigned to luspatercept in the double-blind Treatment Period, reach the maximum treatment duration of 5 years from subjects' Dose 1 Day 1 in the treatment period of this ACE-536-B-THAL-001 study or discontinue earlier and complete the 156 weeks of the Post-treatment Follow-up Period, whichever occurs later; or the date of receipt of the last data point from the last subject that is required for primary, secondary, CCI analyses, whichever is the later date, as pre-specified in the protocol and/or Statistical Analysis Plan.

Study Treatments

Experimental arm. Luspatercept clinical drug product will be provided by the Sponsor as a lyophilized powder in vial. Luspatercept will be administered after reconstitution (see Section 7) as a subcutaneous (SC) injection to subjects by the study staff at the clinical site. Subcutaneous injections will be given in the upper arm, abdomen, or thigh, every 3 weeks during the Treatment Period and during the Long-term Treatment Period, unless dose delay or treatment discontinuation is indicated. Subjects will start luspatercept at 1 mg/kg dose level and can be dose titrated up to a maximum of 1.25 mg/kg.

Control arm. Unblinded designated site personnel at each site will be responsible for preparing the investigational product (see Section 7). Placebo will be administered to subjects as an SC injection by the study staff at the clinical site. Subcutaneous injections will be given in the upper arm, abdomen, or thigh every 3 weeks during the Treatment Period and during the Long-term Treatment Period, unless dose delay or treatment discontinuation is indicated.

Overview of Key Efficacy Assessments

- The primary efficacy assessment will be:
    - Transfusion burden, calculated as units RBC per 12-week period, including the 12 weeks prior to Dose 1 Day 1, Week 13 to Week 24, and Week 37 to Week 48. Additional data obtained for each transfusion will include date, volume in mL, hematocrit of RBCs transfused, and pre-transfusion hemoglobin level.
- Other efficacy assessments will include:
    - Liver iron concentration (LIC, mg/g dw) measured by magnetic resonance imaging (MRI)
    - Daily dose of iron chelation therapy (ICT)
    - Serum ferritin
    - Total hip and lumbar spine bone mineral density (BMD) by dual energy x-ray absorptiometry (DXA)
    - Myocardial iron by MRI
    - Quality of Life using TranQol, SF36
    - Healthcare resource utilization

Overview of Key Safety Assessments

All subjects will be assessed for safety by monitoring adverse events (AEs), clinical laboratory tests, vital signs, electrocardiogram (ECG), cardiac Doppler or Multi Gated Acquisition Scan (MUGA), antidrug antibody (ADA) testing, and Eastern Cooperative Oncology Group (ECOG) performance status.

Overview of Pharmacokinetic Assessments

Population pharmacokinetics will be evaluated, and the relationship between serum drug exposure and clinical endpoints of interest will be explored.

Statistical Methods

The analysis populations for this protocol study are the intent-to-treat (ITT) population and the safety population (definitions for each population are provided in Section 9).

Subjects will be randomized to receive luspatercept or placebo in a 2:1 ratio. A stratified randomization schedule will be implemented. Subjects will be stratified as described above and in Section 3, Overall Study Design.

Based on data from the luspatercept Phase 2 study (A536-04/A536-06), the assumed targeted response rate for the primary endpoint is 40% in the luspatercept group and 20% in the placebo group. A total sample size of 300 (200 in the luspatercept group, 100 in placebo group) will have 90% power to detect the difference between the luspatercept group and the placebo group with a 2-sided alpha of 0.05 and assumed 10% drop-out rate for each treatment group.

Primary Efficacy Analysis The primary efficacy endpoint of this study, erythroid response, is defined as subjects with ≥ 33% reduction from baseline in RBC transfusion burden with a

reduction of at least 2 units from Week 13 to Week 24 compared to the 12-week interval prior to randomization for luspatercept plus BSC versus placebo plus BSC.

The efficacy analysis will be performed on the ITT population. The primary efficacy analysis will be performed based on 24 weeks of data after all subjects have completed the double-blind 24-week treatment period phase or discontinued before reaching 24 weeks of double-blind treatment

The primary endpoint response rate is calculated using the number of responders divided by all subjects in the ITT population. The Cochran Mantel-Haenszel (CMH) chi-square test will be performed with randomization factor as strata and 2-sided type 1 error rate of 0.05.

Secondary Efficacy Analyses The key secondary endpoints will be measured at Week 24 and Week 48 and will be statistically tested in a sequential order at $\alpha = 0.05$ level:

1. Proportion of subjects with erythroid response, defined as ≥ 33% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 37 to Week 48.
2. Proportion of subjects with ≥ 50% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 13 to Week 24.
3. Proportion of subjects with ≥ 50% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 37 to Week 48.
4. Mean change from baseline in transfusion burden from Week 13 to Week 24.

The analyses of secondary efficacy endpoints will be performed on the ITT population. The results will be presented by treatment group. The statistical tests will be conducted to compare the treatment groups.

Detailed description of the other efficacy analyses and safety analysis is provided in Section 9.6.3 and Section 9.7.

Interim Analysis

No interim analysis is planned.

Timing of Analyses:

Clinical Study Report for Marketing Authorization Application

A clinical study report (CSR) for a marketing authorization application (MAA) will include safety and efficacy parameters at the time of the final analysis when all subjects have completed 48 weeks of a double-blind Treatment Period or discontinued before reaching 48 weeks. With this cut-off date and upon database lock, the study will be unblinded. The CSR for MAA will include TEAEs reported for 9 weeks post last dose.

The efficacy analyses included in the CSR for MAA will be conducted on primary, secondary, CCI [redacted], and safety endpoints.

Final Clinical Study Report

The final CSR will include efficacy and safety data at the time of the End of the Trial. (ie, when all subjects initially assigned to luspatercept in the double-blind Treatment Period complete the total treatment duration of 5 years from the subjects' Dose 1 Day 1 in the treatment period of this

ACE-536-B-THAL-001 study or discontinue earlier and complete the 156 weeks of the Post-treatment Follow-up Period, whichever occurs later.)

The final efficacy analyses will be conducted on the primary endpoint, secondary endpoints, and safety endpoints.

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1. INTRODUCTION

This is a Phase 3, double-blind, randomized, placebo-controlled study to determine the efficacy and safety of luspatercept in adults with regularly transfused beta (β)-thalassemia.

1.1. Disease Background - Beta-thalassemia, Pathophysiology, Diagnosis and Treatment

1.1.1. Beta-thalassemia

Beta-thalassemia, one of the most common inherited hemoglobinopathies worldwide, is due to autosomal mutations in the gene encoding β-globin which induce an absence or low-level synthesis of this protein in erythropoietic cells (Weatherall, 2001). About 80 to 90 million people (~ 1.5 % of the global population) are carriers of β-thalassemia with approximately 60,000 symptomatic individuals born annually (Modell, 2007). The annual incidence of symptomatic individuals is estimated at 1 in 100,000 worldwide and 1 in 10,000 in the European Union (EU) (Galanello, 2010). Incidence is highest in the Mediterranean region, the Middle East, and South East Asia (particularly India, Thailand, and Indonesia; this region accounts for approximately 50% of affected births) and incidence is increasing worldwide (eg, Europe, the Americas and Australia) as a result of migration (Colah, 2010; Modell, 2008).

Beta-thalassemia is characterized by a reduction of β-globin chains and a subsequent imbalance in globin chains (α:non-α ratio) of the hemoglobin (Hb) molecule, which results in impaired, or ineffective, erythropoiesis. Complications resulting from ineffective erythropoiesis are described in Section 1.1.2.1. Nearly 200 different mutations have been described in patients with β-thalassemia that affect the β-globin gene, for which patients may be either homozygous or compound heterozygous. Phenotypic findings, therefore, range widely in patients with slight impairment to complete loss of β-globin chain synthesis (Thein, 2013). In addition to deficient β-globin chains, patients may also present with β-thalassemia combined with structural variants of hemoglobin, such as HbE, leading to HbE/β-thalassemia.

Beta-thalassemia comprises a number of different phenotypes with varying severity, including:

- Transfusion-dependent thalassemia (TD): Includes patients with β-thalassemia major or severe forms of β-thalassemia intermedia or HbE/β-thalassemia which require regular red blood cell transfusions
- Non-transfusion dependent thalassemia (NTD): Includes patients with mild-to-moderate β-thalassemia intermedia or HbE/β-thalassemia who may require infrequent transfusions to manage the disease and its complications
- β-thalassemia trait (minor): Heterozygous patients with mild, usually asymptomatic anemia that generally does not require treatment (excluded from the luspatercept target patient population)

At the severe end of the clinical spectrum are patients with β-thalassemia major with symptoms in infancy and reliance on regular red blood cell transfusions for survival. Each year, approximately 23,000 people are born with β-thalassemia major (Weatherall, 2010; Modell, 2008). In contrast, β-thalassemia intermedia patients typically present after the age of 2 years, although accurate global incidence rates have not yet been established (Weatherall, 2001).

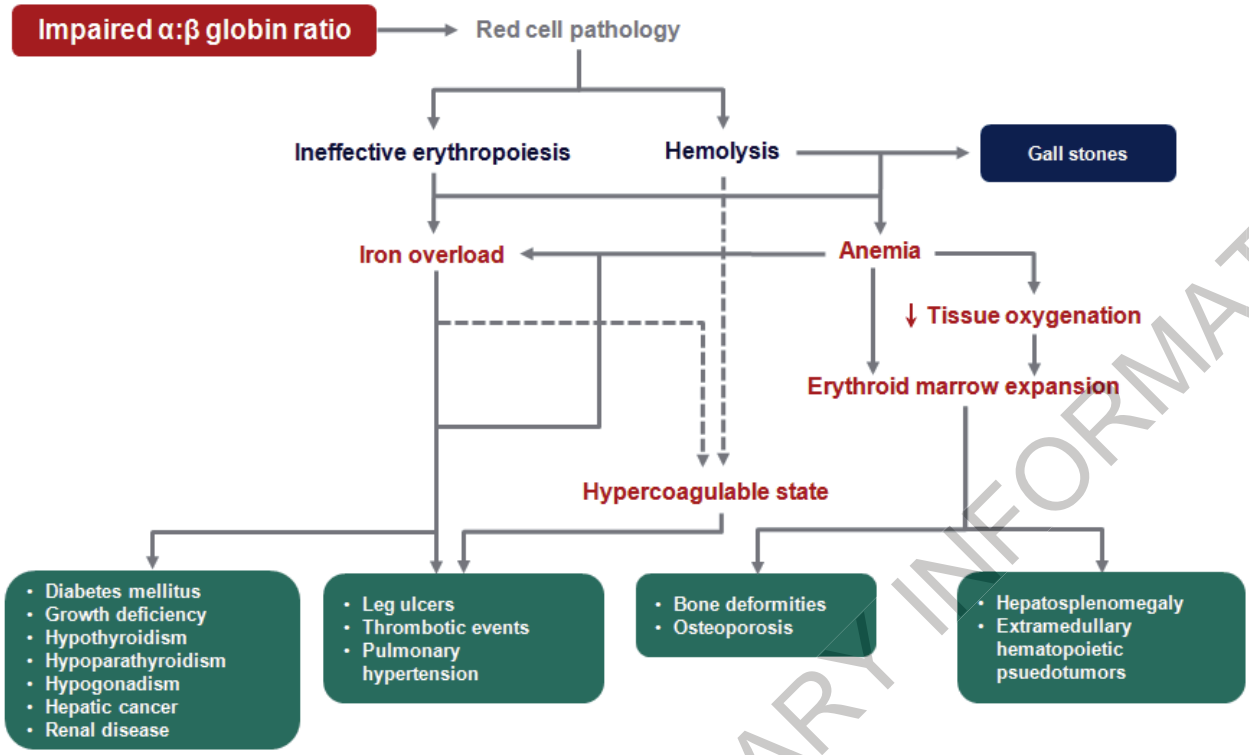
The severity of the disease in β-thalassemia intermedia patients also varies significantly, ranging from mild symptoms at clinical presentation with near normal growth, to a more severe phenotype characterized by early-onset anemia and abnormal physical symptoms including growth and developmental retardation and skeletal deformities. Some β-thalassemia intermedia patients who show growth retardation in childhood or begin to develop clinical complications in adulthood, such as thrombotic events or pulmonary hypertension, can also become transfusion-dependent during their lifetime, regardless of whether they required regular transfusions earlier in life or on presentation (Guidelines for the Management of Non Transfusion Dependent Thalassaemia, 2013). Of note, although β-thalassemia major and intermedia describe patients with different levels of clinical severity, the underlying molecular and pathophysiological basis for the two syndromes are largely similar without intervention (Thein, 2013).

HbE/β-thalassemia is the most common form of thalassemia in India, Bangladesh and Southeast Asia (Colah, 2010), and occurs as a result of co-inheritance of HbE (caused by a substitution in the β-globin gene, leading to the production of structurally abnormal Hb which is synthesized at a reduced rate and behaves like a β-thalassemia allele) and a β^0 allele. Clinically, HbE/β-thalassemia is a highly heterogeneous disease. Typically, anemia with splenomegaly develops from 6 to 12 months of age, with impaired growth during the first decade of the patient's life (Fucharoen, 2012). Clinical symptoms vary widely; patients may be transfusion-dependent or require only occasional or intermittent transfusions. However, these patients are generally managed with red blood cell (RBC) transfusion and iron chelation as needed, although the requirement may differ from other β-thalassemia patients.

1.1.2. Pathophysiology

Ineffective erythropoiesis in individuals with β-thalassemia reflects the consequences of excess, unpaired α-globin (Cao, 2010; Sankaran, 2010). The degree of α-globin to non-α-globin biosynthetic ratio is the major determinate of disease severity, rather than the underproduction of hemoglobin (Weatherall, 1965; Nathan, 1966; Weatherall, 2001). Depending on the β-globin gene defect(s) and their interaction, β-globin production is quantitatively reduced to different extents whereas the synthesis of α-globin continues at normal rates, resulting in an accumulation of excess unmatched α-globin chains in the erythroid precursors. The free α-globin chains are not able to form stable tetramers. Rather, they precipitate in the erythroid precursors forming inclusion bodies that damage the RBC membrane, causing premature destruction (apoptosis) of erythroid precursors in the bone marrow (ineffective erythropoiesis). This leads to a sequence of events resulting in bone marrow expansion, anemia, hemolysis, splenomegaly, extramedullary hematopoiesis, and increased intestinal iron absorption (Figure 1). Any factor that reduces the degree of chain imbalance and the magnitude of α-chain excess, such as coinheritance of α-thalassemia or an innate ability to increase fetal hemoglobin will thus ameliorate the clinical expression of the disease (Cappellini, 2014).

Figure 1: Pathophysiology and Clinical Complications in Beta-thalassemia



Source: Musallam, 2013.

1.1.2.1. Clinical Complications

Eventually all of the pathophysiologic features of β-thalassemia can be linked to the primary imbalance in globin chain production and accumulation of unpaired α-globin chains within the developing erythrocyte, resulting in ineffective erythropoiesis which leads to anemia and a variety of subsequent pathophysiologic mechanisms including hemolysis, iron overload, and hypercoagulability which are in turn linked to clinical morbidities. The severity of the ineffective erythropoiesis drives all clinical complications (Musallam, 2011).

Regularly Transfused (Transfusion-Dependent) β-thalassemia

Regularly transfused β−thalassemia is comprised of two genotypically different but phenotypically similar groups of patients: those with thalassemia major and those with more severe thalassemia intermedia. The major forms of β−thalassemia (also known as Cooley's anemia) are disorders in which life can be sustained only by regular blood transfusions. These conditions usually result from the homozygous or compound heterozygous state for severe β-gene mutations (β^0). Typically, symptoms become manifest during the first year of life, when the normal switch from γ−chains to β−chains does not occur. In some resource-limited settings, the clinical picture in patients who are untreated or poorly transfused, is characterised by growth retardation, pallor, jaundice, poor musculature, genuvalgum, hepatosplenomegaly, leg ulcers, development of masses from extramedullary hematopoiesis, and skeletal changes resulting from expansion of the bone marrow (Guidelines for the Management of Transfusion Dependent Thalassemia, 2014, Galanello, 2010). Many children who are adequately transfused and are fully compliant with iron chelation therapy develop normally, enter puberty and become sexually

mature. The adult population of TD patients suffers from the side effects of chronic transfusions, namely transfusion-associated infections, (particularly hepatitis B and C and in some populations human immunodeficiency virus [HIV]), and organ damage due to iron overload (including liver, heart, and endocrine glands) (Galanello, 2010; Cappellini, 2014).

Some β-thalassemia intermedia patients who show growth retardation in childhood or begin to develop clinical complications in adulthood, such as thrombotic events or pulmonary hypertension, may also require regular transfusions and become transfusion-dependent during their lifetime, regardless of whether they required regular transfusions earlier in life or on presentation (Guidelines for the Management of Non Transfusion Dependent Thalassaemia, 2013).

The primary cause of death in adult TD patients remains cardiac events due to iron overload mainly caused by RBC transfusions (Rund, 2005; Voskaridou, 2012) although recent studies show that liver disease is also becoming a leading cause of morbidity and mortality (Voskaridou, 2012).

Many aspects of heart failure in these patients are still poorly understood. It is clear that it is multifactorial involving chronic anemia, iron overload, myocarditis, pericarditis and probably other mechanisms. Furthermore, genetic modifiers acting at a tertiary level may impact the complications of thalassemia (Cappellini, 2014).

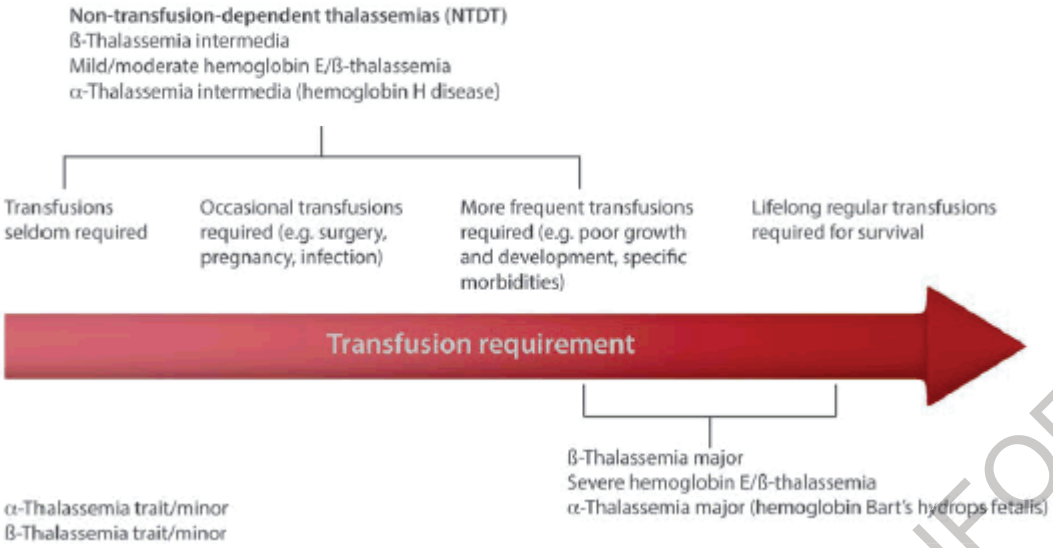
1.1.2.2. Diagnosis of Beta-thalassemia

Beta-thalassemia include three forms of increasing severity. Beta-thalassemia minor (or trait) is due to the inheritance of one mutant β-globin gene and may result in microcytic anemia. The other two conditions, β-thalassemia intermedia and β-thalassemia major, are clinically relevant requiring medical management. The most severe form, β-thalassemia major, results from homozygosity or compound heterozygosity for a mutant β-globin allele (β^0/β^0, β^+/β^+, β^0/β^+, β^0/HbE, β^+/HbE). Beta-thalassemia intermedia phenotype can result from compound heterozygosity of milder mutations (β^0 or β^+, as well as with Hb variants including HbE), or coinheritance of two severe β mutations with either α-thalassemia or determinants that increase fetal hemoglobin production. Patients who are heterozygous for a β-globin mutation can have symptomatic thalassemia if they co-inherit duplication of the α-globin gene(s). The diagnosis of β-thalassemia can be confirmed by the presence of the β-thalassemia trait in both parents (Thein, 2013).

Beta-thalassemia major and β-thalassemia intermedia have no specific molecular correlate but rather encompass a wide spectrum of clinical and laboratory abnormalities. Patients referred to as having β-thalassemia major are usually those who come to medical attention in infancy and subsequently require regular transfusions to survive. Those who present with symptoms later in their life or who seldom need transfusions early in life are said to have β-thalassemia intermedia. Hematologic findings include microcytic, hypochromic anemia and elevated HbA2 and fetal hemoglobin [HbF]. Note that β-thalassemia intermedia patients can present as a severe phenotype and may require regular transfusions later in life in order to better manage certain complications of the disease.

The spectrum of transfusion requirements in various thalassemia forms is illustrated in Figure 2 below (Musallam, 2013):

Figure 2: Transfusion Requirement in Thalassemia



1.1.2.3. Treatment of Beta-thalassemia

Current treatment options for β-thalassemia are limited; transfusions remain the mainstay of treatment. In regularly transfused patients, iron chelation therapy is required to treat and/or prevent transfusional iron overload-related complications. In non-transfusion dependent patients, the treatment strategy is focused on iron overload-related complications, as well as complications related to the disease itself. An overview of treatment options for β-thalassemia is presented below.

1.1.2.3.1. Transfusion

As mentioned, β-thalassemia transfusion-dependent patients require regular transfusions, which can result in a number of subsequent complications (Guidelines for the Management of Transfusion Dependent Thalassemia, 2014). These include infections, iron overload and related complications, such as cardiac, liver and endocrine problems, and ultimately, death due to organ failure. In addition, alloimmunization may occur, whereby the recipient mounts an immune response to donor antigens, resulting in various clinical consequences. Alloimmunization is more common in children who begin transfusion therapy after 1 to 3 years of age than in those who begin transfusion therapy earlier. Some evidence also suggests that new alloantibodies develop more frequently after splenectomy (Thompson, 2011). The use of extended antigen matched donor blood is effective in reducing the rate of alloimmunization.

1.1.2.3.2. Iron Overload and Iron Chelation Therapies

In patients with β-thalassemia requiring regular transfusions, iron overload occurs mainly as a result of accumulation of iron from transfusions and, to a lesser extent, increased intestinal absorption. In contrast, in non-transfusion dependent β-thalassemia patients, non-transfusional iron overload is present due to increased intestinal absorption of iron secondary to ineffective erythropoiesis and suppression of hepcidin (Gardenghi, 2007; Ginzburg, 2011).

The main goal of iron chelation therapy is to maintain safe levels of body iron at all times. Once iron overload has occurred, removal of stored iron is slow and inefficient, because only a small proportion of body iron is available for chelation at any given time (Guidelines for the Management of Transfusion Dependent Thalassemia, 2014).

The standard chelation therapy for more than 40 years was deferoxamine (DFO). Unfortunately, compliance with the rigorous requirements of daily subcutaneous infusions was a serious limiting factor and in noncompliant patients life expectancy was not different from that in the pre-DFO era (Cappellini, 2014, Gabutti, 1996). Subsequently, 2 oral iron chelators have been marketed including: deferiprone (DFP) and deferasirox (DFX). A potential benefit of combined DFO/DFP therapy has been observed for patients with very high levels of cardiac iron (Cappellini, 2014, Tanner 2007).

Deferasirox (DFX) has been shown to be safe and effective in removing excess iron from different organs including the heart (Cappellini, 2014). However some patients do not respond to DFX at maximally tolerated doses. Deferasirox use is individualized according to age and compliance history to previous chelation, and monitored using ferritin levels, and measurements of cardiac and liver iron by MRI (Guidelines for the Management of Transfusion Dependent Thalassemia, 2014).

1.1.2.3.3. Splenectomy

Hypersplenism may occur as a result of large numbers of cells being pooled and destroyed in the spleen's reticulo-endothelial system, and hemodilution because of an increased plasma volume. Therefore, spleen size is carefully monitored in all patients with β-thalassemia (Cappellini, 2014a). Many patients with β-thalassemia major require splenectomy. Good clinical management may delay or prevent hypersplenism, reducing the need for splenectomy (Piga, 2011). As abnormalities of platelets and red blood cells become more prominent following splenectomy, patients are at an increased risk of thrombotic and vascular events, with an associated increased mortality rate. (Cappellini, 2000; Taher, 2006).

1.1.2.3.4. Fetal Hemoglobin (HbF) Induction

Fetal hemoglobin is made up of alpha and gamma chains and is the predominant hemoglobin in RBCs of patients with severe β-thalassemia. Increasing levels of fetal hemoglobin (HbF) can theoretically ameliorate the severity of β-thalassemia by reducing the amount of free alpha-globin.

Hydroxyurea (or hydroxycarbamide) is the fetal hemoglobin inducer for which most data in NTD subjects have been generated (Guidelines for the Management of Non Transfusion Dependent Thalassaemia, 2013). Hydroxyurea use in TD subjects has never been systematically studied (Guidelines for the Management of Transfusion Dependent Thalassemia, 2014). The exact mechanisms by which hydroxyurea induces fetal hemoglobin production are not fully understood. A cytotoxic effect resulting in stress erythropoiesis with increased fetal hemoglobin levels is most commonly proposed (Mabaera, 2008). However, such cytotoxic agents are associated with toxicity with long-term use, and are potentially mutagenic.

There is a lack of randomized clinical trials investigating the efficacy of hydroxyurea treatment on decreasing transfusion burden and it is not approved to treat β-thalassemia. Though data are available from a large number of single-arm trials or retrospective analyses of hydroxyurea

therapy, patient numbers are small and results have not been consistently reproduced (Musallam 2013; Cappellini, 2014a).

1.1.2.3.5. Hematopoietic Stem-Cell Transplantation

Early, allogeneic hematopoietic stem cell transplantation (HSCT) has curative potential. In adult patients who received allogeneic HSCT from human leukocyte antigen (HLA)-identical donors, the curative potential is reported to be from approximately 60% (Olivieri, 1999) to 73% (Angelucci, 2008). However, this option is limited by availability of appropriate donors and by risks associated with the bone marrow transplant procedure. Additionally, HSCTs are more commonly performed in patients under 21 years of age (preferably under 16 years) than in older patients (Sabloff, 2011).

1.1.2.3.6. Erythropoiesis-stimulating Agents (ESA)

Erythropoiesis-stimulating agents are not indicated for and are not commonly used for the treatment of β-thalassemia. The anemia and resulting hypoxia in β-thalassemia leads to already increased in serum erythropoietin (EPO) levels in an attempt to compensate for the reduced oxygen carrying capacity. As EPO binds to receptors on the earliest erythroid progenitors, the use of ESAs may further increase erythroid mass and the production of abnormal RBCs (Fibach, 2014). Thus, treatment with ESAs is associated with potential risks, including erythroid expansion, increased iron absorption, and extramedullary hematopoiesis (Rachmilewitz, 1998).

1.1.2.3.7. Summary of Current Treatments

Transfusions are still the mainstay of therapy. Transfusions are associated with iron overload and other complications. Transfusion therapy leads to poor quality of life (QoL) due to time lost, and it is associated with costs and requirement for iron chelation therapies (which are also associated with adverse events). Hematopoietic stem cell transplantation from an identical family donor is an alternative treatment option for children with thalassemia and the only one which can result in cure (SCCCAT, 2008). However, this option is limited by availability of appropriate donors and by risks associated with the bone marrow transplant procedure.

Other than iron chelation agents with indications for the treatment of chronic iron overload, there is no approved drug therapy specifically for the treatment of patients with β-thalassemia. Additionally, there is limited use of currently available pharmacological therapies. For example, cytotoxic agents can inhibit erythroid proliferation, but are associated with toxicity with long-term use, and are potentially mutagenic. As such, hydroxyurea is rarely used for the chronic treatment of β-thalassemia. ESAs have minimal therapeutic and may pose additional risks to β-thalassemia patients. Patients require many other therapies to treat the consequence of anemia such as folic acid supplements and medication for osteoporosis.

Given the current lack of safe and effective drug treatments, there is significant unmet medical need for the development of new therapies that specifically address the underlying pathophysiology of β-thalassemia including ineffective erythropoiesis and anemia, to decrease transfusions and prevent associated complications.

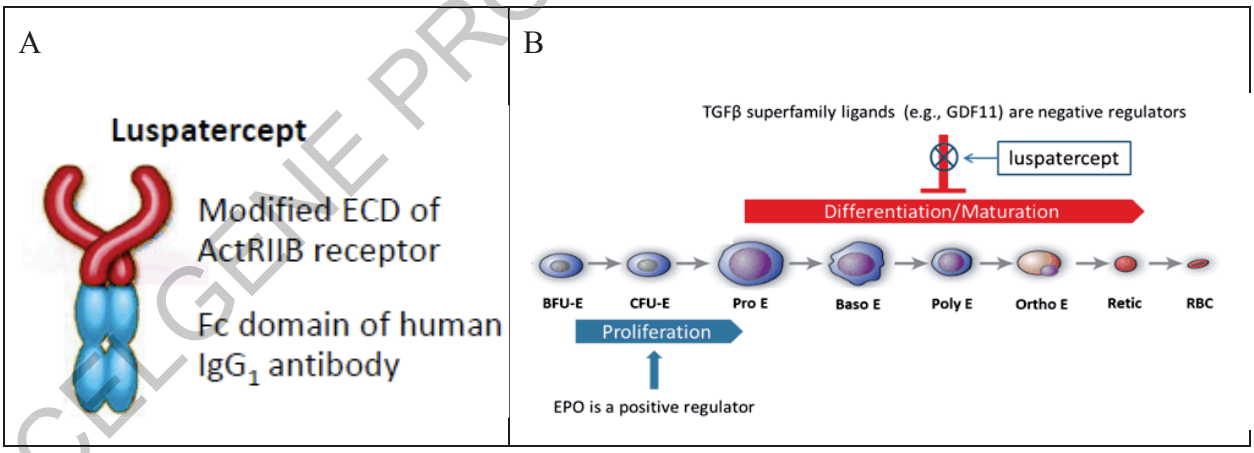
1.2. Compound Background - Luspatercept

Luspatercept (ACE-536) is a recombinant fusion protein consisting of a modified form of the extracellular domain (ECD) of the human activin receptor type IIB (ActRIIB) linked to the human immunoglobin G 1 (IgG1) Fc domain (Figure 3A). The ActRIIB receptor and its ligands are members of the transforming growth factor-β (TGF-β) superfamily, a group of proteins involved in the development, differentiation, and/or maturation of various tissues. No species differences have been described in the ligand-receptor interactions among members of the TGF-β family as the ligands and receptors are highly conserved across species (Massague, 1998). Thus, observations from pharmacology studies of luspatercept or its murine ortholog RAP-536 in animal models provide significant insight into the potential of luspatercept to treat human disease.

Members of the TGF-β ligands through their binding to activin receptors, are involved in modulating the differentiation of late-stage erythrocyte precursors (normoblasts) in the bone marrow. In nonclinical experiments, luspatercept has been shown to bind with high affinity to some TGF-β ligands (eg, GDF11, GDF8, BMP6 and activin B) CCI [redacted]. The mechanism of action of luspatercept is independent from that of EPO (Suragani, 2014a). While EPO stimulates proliferation and differentiation of early erythroid progenitors, luspatercept promotes stimulation of the later, maturation phase of erythroblast differentiation and maturation in the bone marrow (Figure 3B and Section 1.3).

During normal erythropoiesis, GDF11 appears to inhibit differentiation and maintain the survival of immature erythroid progenitors, but its expression is decreased as cells mature, and thus its effect is transient. In the thalassemia model, defects in erythroid differentiation led to an accumulation of GDF11 expressing cells that maintained their own survival (Dussiot, 2014). Recent studies (Dussiot, 2014; Suragani, 2014a) identified GDF11 as a regulator of erythropoiesis and showed that its inhibition in mouse models of anemia with ineffective erythropoiesis restores normal erythropoietic differentiation and improves anemia.

Figure 3: Luspatercept Schematic Representation and Mechanism of Action



1.2.1. Summary of Nonclinical Studies with Luspatercept

A brief summary of key findings from pharmacology and toxicology studies is provided below. Please refer to the Investigator's Brochure (IB) for detailed information concerning the available

pharmacology, toxicology, drug metabolism, clinical studies, and adverse event profile of the investigational product (IP). The most recent version of the luspatercept IB should be reviewed prior to initiating the study.

1.2.1.1. Pharmacology Studies

In vitro and in vivo nonclinical pharmacology studies have been conducted with luspatercept or its murine ortholog, RAP-536. The RAP-536 molecule has the same ECD as luspatercept, but contains a murine IgG Fc domain in place of the human IgG Fc. The RAP-536 ortholog is intended to be less immunogenic in rodent species and therefore permits the conduct of longer term pharmacology studies in rodents without the confounding influence of immune reactivity (Suragani, 2014a).

The Hbb major-/- transgenic mouse was used to test RAP-536 in a model of β-thalassemia. This model represents human β-thalassemia intermedia, which is characterized by severe anemia, failure of erythroid differentiation, increased apoptosis of erythroid precursors, erythroid hyperplasia, splenomegaly and iron overload. Treatment with RAP-536 led to significant improvement in hematological parameters, including RBC and hemoglobin. Further effects of treatment included decreased reticulocytes, red cell distribution width, EPO, bilirubin, RBC inclusion bodies, reactive oxygen species, serum ferritin, liver iron concentration (LIC), and spleen size, as well as increased hepcidin and bone mineral density, and improvements in RBC morphology (Suragani, 2014).

Additional information regarding the pharmacological effects of luspatercept is summarized in the current version of the luspatercept IB.

CCI [redacted]

1.2.1.3. Summary of Clinical Experience

One Phase 1 trial with healthy postmenopausal women has been completed (Attie, 2014). Two Phase 2 studies with β-thalassemia are ongoing:

- Study A536-04 is entitled "A Phase 2, Open-Label, Ascending Dose Study to Evaluate the Effects of ACE-536 in Patients with β-Thalassemia".
- Study A536-06 is entitled "An Open-Label Extension Study to Evaluate the Long-Term Effects of ACE-536 in Patients with β-Thalassemia Previously Enrolled in Study A536-04".

CCI [redacted]

CCI [redacted] A reduction (> 33%) in RBC transfusion burden was observed in the majority of the transfusion-dependent β-thalassemia subjects. Luspatercept was well tolerated at the tested dose levels (0.2 to 1.25 mg/kg SC every three weeks).

Additional information regarding clinical experience with luspatercept is summarized in the current version of the luspatercept IB.

1.2.1.4. Potential Risks of Human Use

Increases in hematologic parameters (RBC, hemoglobin, hematocrit, reticulocytes) are expected pharmacologic effects of luspatercept treatment. Increases in systolic and diastolic blood pressures may occur in concert with increases in hemoglobin values. Excessive or rapid increases in hemoglobin or blood pressure may occur and will be monitored. Dose modifications rules for individual subjects and dose titration/reduction rules will be utilized to minimize risks associated with increased RBC parameters.

Adverse events considered probably or possibly related to study drug that were reported in the Phase 1 study in healthy volunteers included injection site hemorrhage and injection site macule. As of 07 July 2016, adverse events reported regardless of causality in the ongoing Phase 2 studies in MDS and β-thalassemia included bone pain, headache, asthenia, myalgia, arthralgia, nasopharyngitis, pyrexia, oropharyngeal pain, diarrhea, fatigue, musculoskeletal pain, cough and influenza. CCI [redacted]

CCI [redacted] Luspatercept must not be given to a pregnant woman or a woman who intends to become pregnant. If a woman becomes pregnant while taking luspatercept, the medication must be stopped immediately.

If luspatercept is taken during pregnancy, a teratogenic effect in humans cannot be ruled out. In addition, since it is unknown if luspatercept is excreted in breast milk, breastfeeding is prohibited in all protocols.

CCI [redacted]

As with all biologics, there is the potential for antidrug antibodies (ADA) that can be associated with increased drug clearance and hypersensitivity reactions. CCI [redacted]

Please refer to the Investigator's Brochure (IB) for additional information regarding findings from toxicology studies. CCI [redacted] Safety will be monitored closely through adverse event (AE) reporting, clinical laboratory tests, vital signs, and physical examinations, and ongoing review of unblinded data by an external DMC.

A comprehensive review of luspatercept, as well as details regarding the information summarized above, is provided in the IB. The most recent version of the luspatercept IB should be reviewed prior to initiating the study.

1.3. Rationale

1.3.1. Study Rationale and Purpose

In β-thalassemia, the imbalance in globin chain production and accumulation of unpaired α-globin chains within the developing erythroblast results in ineffective erythropoiesis, which leads to anemia. The unbound free α-globin chains precipitate in erythroid precursors and these precipitates are associated with the production of reactive oxygen species (ROS), which induce the death of erythroid precursors at the polychromatophilic stage (Weiss, 2009; Ribeil, 2013), and thus results in a suboptimal production of mature RBCs (ineffective erythropoiesis), a hallmark of β-thalassemia (Ginzburg, 2011). It has been proposed that members of the TGF-β superfamily participate in the proliferation and differentiation of erythroid progenitors (Dussiot, 2014).

It has been demonstrated that RAP-536 (the murine ortholog of luspatercept), a modified ActRIIB ligand trap, can alleviate anemia in a transgenic mouse model of β-thalassemia (Hbb th1/th1 mice) in part by promoting differentiation of later-stage erythroid precursors and reducing hemolysis, effects likely due to reduced α-globin aggregates in erythrocytes and decreased ROS in erythroid precursors and peripheral erythrocytes. In addition to improving these fundamental features of the disease, RAP-536 treatment in Hbb th1/th1 mice produced beneficial effects on several interrelated disease complications, such as decreased reticulocytes, red cell distribution width, EPO, bilirubin, RBC inclusion bodies, reactive oxygen species, serum ferritin, LIC, and spleen size, as well as increased hepcidin and bone mineral density, and improvements in RBC morphology (Suragani, 2014).

Interim data from the ongoing Phase 2 clinical trial with luspatercept in β-thalassemia subjects (A536-04) demonstrated that 75% of subjects treated with luspatercept for 3 months at dose levels of 0.8 to 1.0 mg/kg met the primary efficacy endpoint, notably (i) increase in hemoglobin ≥ 1.5 g/dL for ≥ 2 weeks in 50% of NTD subjects, and (ii) decrease in RBC transfusion burden ≥ 20% (median 67%, ranging from 43 to 100%) in 100% of evaluable TD subjects. In addition, liver iron concentration and serum ferritin decreased in both TD and NTD subjects, and rapid healing of leg ulcers was observed in 3 of 3 subjects. Furthermore, the safety profile was favorable with no related serious adverse events. These data strongly support further evaluation of luspatercept in subjects with β-thalassemia.

The standard of care for transfusion-dependent β-thalassemia subjects involves regular blood transfusions, usually administered every two to four weeks, to maintain a pre-transfusion hemoglobin level of 9 to 10.5 g/dL, for the purpose of suppressing the bone marrow. There is no approved drug therapy specifically for the treatment β-thalassemia. Iron chelation agents are approved for the treatment of chronic iron overload which is primarily due to transfusions in transfusion-dependent patients for the treatment of chronic iron overload.

Considering the current lack of safe and effective drug therapies, this regularly-transfused population would benefit from an active drug that specifically addresses the underlying pathophysiology of β-thalassemia including ineffective erythropoiesis, and anemia, and to reduce the dependence on transfusion therapy, thus reducing both the risk of complications from β-thalassemia and transfusions therapy, including iron overload.

Luspatercept has the potential to provide benefit in a variety of conditions in which ineffective erythropoiesis contributes significantly to anemia and overall disease morbidity, including β-thalassemia. The ability of luspatercept to rapidly increase and sustain Hb concentrations in anemic patients supports the clinical development of luspatercept for the treatment of patients with anemia associated with β-thalassemia.

1.3.2. Rationale for the Study Design

The current study is a Phase 3, multicenter, randomized, double-blind, placebo-controlled, study to compare the efficacy and safety of luspatercept plus BSC versus placebo plus BSC in adult subjects with regularly transfused β-thalassemia. Subjects are required to be diagnosed with β-thalassemia (including Hemoglobin E/β-thalassemia), aged ≥ 18 years and be regularly transfused. Transfusion dependence is defined in this study as receiving 6-20 RBC units during the 24 weeks prior to randomization and no transfusion-free period for > 35 days. The minimum of 6 units has been defined based on potential transfusion practices, including a minimum of 1 unit every 4 weeks (or 6 units/24 weeks), which may be observed in countries with limited blood supply, as per feedback received from investigators. The maximum of 20 units in the 24 weeks prior to randomization was determined based on the clinical data from Phase 2 studies with luspatercept in β-thalassemia, in which no subjects with transfusion burden >20 units/24 weeks achieved the planned primary endpoint for response in this Phase 3 study.

The multicenter nature of the study provides assurance that the results are likely to have general applicability. The design of this study (ie, randomized, double-blinded, placebo-controlled, and parallel-group) will eliminate bias in assignment of the IP or in data interpretation.

Given the lack of agents globally approved and/or used in β-thalassemia to attenuate ineffective erythropoiesis and/or to correct anemia, the standard of care is largely supportive with RBC transfusion and splenectomy. There may be some subjectivity with regard to deciding when and how much to transfuse a patient in a particular clinical situation. For this reason, the Sponsor plans to use saline-placebo control as the comparator in the proposed study in regularly transfused β-thalassemia subjects, as well as implement clear guidelines regarding when to administer transfusions while on treatment, based on the pre-treatment transfusion history for each patient (refer to Section 8.1.4).

There will be unblinded designated site personnel at each site responsible for preparing the IP. All other site personnel, including the Investigators and monitors, will be blinded to treatment assignment during the study.

Best supportive care is not a treatment regimen in this study. However, it may be used in combination with the investigational product as deemed necessary. Best supportive care in both treatment arms may include RBC transfusion, iron-chelating agents, use of antibiotic, antiviral and antifungal therapy, and/or nutritional support, thus the risk of not providing subjects with appropriate care is minimized.

1.3.2.1. Rationale for the Primary Endpoint

The primary endpoint of the study is the proportion of subjects with erythroid response, defined as ≥ 33% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 13 to Week 24. To mitigate any potential bias should subjects in the control arm drop

out early due to lack of response, the primary efficacy analysis will be measured at 13-24 weeks. Importantly, subjects will continue to be followed on their assigned treatment, in a blinded manner, for 48 weeks (see Section 3) to generate longer-term efficacy and intermediate term safety information.

A 33% or greater reduction in transfusion burden is considered to be clinically meaningful for patients regularly transfused based on the decrease in the transfusional iron accumulation and related complications. We have estimated that a patient who requires 2 RBC units every 4 weeks pretreatment and reduced frequency to 2 units every 6 weeks will benefit from a reduction in transfusional iron intake of approximately 1700 mg/year, based on an estimated 200 mg iron/RBC unit (Cohen, 2008; Porter, 2001). In a 50 kg patient, this reduction of transfusional iron of 1700 mg/year would translate into a reduction of liver iron concentration of ~3 mg/g dry weight (Angelucci, 2000), which has been considered a clinically meaningful change in recent iron chelation studies in β-thalassemia populations (Cappellini, 2006; Taher, 2012). Reduced transfusional iron can also lead to empirical reduction in iron chelation therapy dose (Cohen, 2008), thus reducing the risks and costs associated with that therapy.

To further evaluate the benefit for patients, we have included as a key secondary endpoints a ≥ 50% reduction in RBC transfusion burden at 13-24 weeks. To address the durability of response, secondary endpoints are including that evaluate reduction in transfusion burden measured at 37-48 weeks of treatment.

Reduced survival in regularly transfused thalassemia patients is largely due to iron overload complications in major organs, in particular involving the heart and liver. In addition, blood transfusion exposes patients to a variety of risks (eg, alloimmunization, infection), and may be affected by limited access to safe blood products in developing countries, where the majority of the β-thalassemia patients reside. These present major challenges in managing patients requiring regular/frequent transfusions. Therefore, reduction in transfusion burden is expected to provide numerous benefits for these patients, including improvement in quality of life and a reduction in the need for iron chelation therapy.

Clear guidelines regarding when to alter either the frequency or number of RBC units transfused are specified in the protocol, with the goal of maintaining or improving the previously established pre-transfusion hemoglobin threshold for each patient (refer to Section 8.1.4). This will decrease the subjectivity with respect to transfusions and provide an accurate measure of change in transfusion requirement.

1.3.2.2. Rationale for the Secondary Endpoints

The proposed secondary endpoints, including safety, other measures of efficacy and pharmacokinetics, are included in Section 2.

Safety Assessments:

Safety will be assessed by evaluation of AEs and laboratory data. Adverse events and abnormal laboratory value severity will be graded using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) (version 4.0, current active minor version). All grades and Grade 3/4 treatment-emergent adverse events (TEAEs), related to investigational product, and serious TEAEs will be summarized separately.

Reduction in Transfusion Burden by ≥ 33% From Baseline From Week 37 to Week 48:

Subjects will be followed for 48 weeks in a blinded manner to generate longer-term efficacy data.

Reduction in Transfusion Burden by ≥ 50% From Baseline:

To further evaluate the benefit for subjects, key secondary endpoints will include a ≥ 50% reduction in RBC transfusion burden for weeks 13-24, as well as for weeks 37-48.

Iron Overload:

In β-thalassemia patients who are regularly transfused, iron overload occurs mainly as a result of accumulation of iron from transfusions and, to a lesser extent, increased intestinal absorption of iron due to hepcidin suppression.

It is generally accepted that LIC is the most reliable indicator of body iron overload (Guidelines for the Management of Transfusion Dependent Thalassaemia, 2014) and an indicator of risk for cardiac iron overload. Normal LIC values are up to 2 mg/g dry weight (dw). Patients with LIC of ≥ 3 mg/g dw are considered to have iron overload (Guidelines for the Management of Non Transfusion Dependent Thalassaemia, 2013; Guidelines for the Management of Transfusion Dependent Thalassaemia, 2014). At ≥ 7 mg/g dw there is an increased risk of iron induced complications; ≥ 15 mg/g dw is associated with an increased risk of cardiac disease and early death in thalassemia patients with transfusional iron overload (Olivieri, 1997). A 20% reduction is considered clinically meaningful and a 30% decrease over 1 year was agreed to be clinically relevant by the study Steering Committee for the Thalassa trial for iron chelation therapy (Taher, 2010).

Iron Chelation Therapy Use:

Iron chelation therapy (ICT) is essential to survival of heavily transfused patients; however, it is frequently associated with adverse events. It has been reported that compliance with the rigorous requirements of daily subcutaneous infusions of deferoxamine (DFO) was a serious limiting factor and that life expectancy for noncompliant patients was not different from that in the pre-DFO era (Cappellini, 2014, Gabutti, 1996).

Although two oral iron chelators are now available in various regions, deferiprone (DFP) and deferasirox (DFX), some patients do not respond to treatment at maximally tolerated doses (Guidelines for the Management of Transfusion Dependent Thalassaemia, 2014). Reducing transfusion burden will very likely reduce ICT daily dosage requirements and possibly the need for ICT at all, which will provide a number of benefits to the patients.

Quality of Life:

TranQol and Short Form (SF)-36, questionnaires will be used to assess the impact of the treatment on the quality of life in patients with β-thalassemia. SF-36 has been used in several prior studies in the β-thalassemia population and is well known to Investigators from multiple countries. TranQol has been developed for and validated in patients with transfusion-dependent β-thalassemia (Klaassen, 2014). It contains questions that specifically pertain to QoL issues related to transfusion burden in thalassemia patients, such as impact on school or work activities.

Healthcare Resource Utilization:

The economic objective of this study is to characterize medical resource utilization among subjects treated with luspatercept as compared to subjects receiving placebo treatment. To facilitate this aim, medical healthcare resource utilization (HRU) data will be collected. In addition, the number of transfusion events will be evaluated, as these relate directly to hours or days devoted to receiving RBC transfusions that can impact patients' quality of life.

1.3.3. Rationale for Dose, Schedule and Regimen Selection

The starting dose level of 1 mg/kg and the maximum dose level of 1.25 mg/kg are based on clinical data from the ongoing Phase 2 A536-04 and A536-06 studies, where the increase in hemoglobin from baseline was maintained better with 1.0 mg/kg compared to lower dose levels, and 1.25 mg/kg was found to be safe and well tolerated.

Selection of the dosing schedule is based on the observed duration of the luspatercept effect on hemoglobin response as well as pharmacokinetic parameters for luspatercept in β-thalassemia patients. CCI [redacted]

Additional information regarding these clinical studies is summarized in the current version of the luspatercept IB.

CCI [redacted]

2. STUDY OBJECTIVES AND ENDPOINTS

Table 1: Study Objectives

Primary Objective
The primary objective of the study is: • To determine the proportion of subjects treated with luspatercept plus BSC versus placebo plus BSC who achieved erythroid response, defined as ≥ 33% reduction from baseline in transfusion burden (units RBCs / time) with a reduction of at least 2 units, from Week 13 to Week 24.
Secondary Objective(s)
The secondary objectives are: • To evaluate the proportion of subjects who achieve ≥ 33% reduction from baseline in transfusion burden from Week 37 to Week 48 versus placebo • To evaluate the proportion of subjects who achieve ≥ 50% reduction from baseline in transfusion burden from Week 13 to Week 24 versus placebo • To evaluate the proportion of subjects who achieve ≥ 50% reduction from baseline in transfusion burden from Week 37 and Week 48 versus placebo • To evaluate the mean change from baseline in transfusion burden from Week 13 to Week 24 • To evaluate the mean change from baseline in liver iron concentration (LIC) versus placebo • To evaluate the mean change from baseline in mean daily dose of iron chelation therapy (ICT) used versus placebo • To evaluate the mean change from baseline in serum ferritin versus placebo • To evaluate the effect of luspatercept on osteoporosis/osteopenia, total hip and lumbar spine measured by bone mineral density versus placebo • To evaluate mean change from baseline in myocardial iron versus placebo • To evaluate mean change from baseline in QoL assessments, such as TranQol and SF-36, versus placebo • To evaluate the effect of luspatercept on healthcare resource utilization versus placebo • To evaluate the proportion of subjects who are transfusion independent for ≥ 8 weeks versus placebo • To evaluate the duration of reduction in transfusion burden or transfusion independence • To evaluate the time to erythroid response • To evaluate the post-baseline transfusion events frequency versus placebo • To evaluate the population pharmacokinetics (PK) of luspatercept in subjects with β-thalassemia • To evaluate the safety and immunogenicity of luspatercept versus placebo
CCI [redacted]
[redacted]

Table 2: Study Endpoints

Endpoint	Name	Description	Time of Endpoint Measurement			Timeframe
			24 weeks	48 weeks	Long -Term	
Primary Endpoint	• Proportion of subjects with hematological improvement, defined as ≥ 33% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 13 to Week 24 compared to the 12-week interval prior to randomization for luspatercept plus BSC versus placebo plus BSC.	Number of RBC units transfused from Week 13 to Week 24, and in the 12 weeks prior to randomization	X	-	-	12 weeks prior to randomization; Week 13-Week 24.
Safety Endpoints	• Safety, including: – Type, frequency, and severity of adverse events and relationship to luspatercept (per NCI CTCAE version 4.0)	Frequency/severity of adverse events	X	X	X	All AE Dose 1 Day 1 through up to 9 weeks post last dose. Only related AEs from week 9 until End of Study.
	• Frequency of antidrug antibodies CCI [redacted]	ADA measurements; CCI [redacted]	X	X	X	Dose 1 Day 1 through up to 2 years
Secondary Efficacy Endpoints	• Proportion of subjects with hematological improvement, defined as ≥ 33% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 37 to Week 48 compared to the 12-week interval prior to randomization for luspatercept plus BSC versus placebo plus BSC.	Number of RBC units transfused from Week 37 to Week 48, and in the 12 weeks prior to randomization	-	X	-	12 weeks prior to randomization; Week 37-Week 48

Table 2: Study Endpoints (Continued)

Endpoint	Name	Description	Time of Endpoint Measurement			Timeframe
			24 weeks	48 weeks	Long -Term	
Secondary Efficacy Endpoints	• Proportion of subjects with ≥ 50% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 13 to Week 24 compared to the 12-week interval prior to randomization for luspatercept plus BSC versus placebo plus BSC	Number of RBC units transfused from Week 13 to Week 24, and in the 12 weeks prior to randomization	X	-	-	12 weeks prior to randomization; Week 13-Week 24
	• Proportion of subjects ≥ 50% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 37 to Week 48 compared to the 12-week interval prior to randomization for luspatercept plus BSC versus placebo plus BSC	Number of RBC units transfused from Week 37 to Week 48, and in the 12 weeks prior to randomization	-	X	-	12 weeks prior to randomization; Week 37-Week 48
	• Mean change from baseline in transfusion burden (RBC units) from Week 13 to Week 24	Change from baseline as continuous variable	X	-	-	12 weeks prior to randomization; Week 13-Week 24
Other Efficacy Endpoints	• Mean change from baseline in liver iron concentration (LIC, mg/g dw) by MRI	LIC by MRI	X	X	X	Screening, as per Table 3, up to last MRI for LIC visit
	• Mean change from baseline in mean daily dose of ICT	Daily dose of ICT	X	X	X	12 weeks prior to first IP treatment; last 12 weeks of the 48-week double-blind Treatment Period or last 12 weeks of study treatment if discontinue early.
	• Mean change from baseline in serum ferritin	Serum ferritin	X	X	X	4 weeks prior to first IP treatment; last 12 weeks of the 48 weeks double-blind Treatment Period or last 12 weeks of study treatment if discontinue early.

Table 2: Study Endpoints (Continued)

Endpoint	Name	Description	Time of Endpoint Measurement			Timeframe
			24 weeks	48 weeks	Long -Term	
Other Efficacy Endpoints	• Mean change from baseline in total hip and lumbar spine bone mineral density by DXA	Total hip and lumbar spine BMD by DXA	X	X	X	Screening up to last DXA scan visit
	• Mean change from baseline in myocardial iron by MRI	Myocardial iron by T2* MRI	-	X	X	Screening, as per Table 3, up to last MRI for T2* measurement visit.
	• Mean change in Quality of Life measures	Self-reported QoL assessed by TranQoL, and SF-36 questionnaires	X	X	X	Screening, as per Table 3, up to last QoL measurement visit.
	• Healthcare resource utilization	Medical resource utilization (refer to Section 9.6.3.7)	X	X	X	Screening until End of Study
	• Proportion of subjects who are transfusion independent for ≥8 weeks during treatment	RBC transfusions	X	X	X	Dose 1 Day 1 through 3 weeks post last dose
	• Duration of reduction in transfusion burden	RBC transfusions (refer to Section 9.6.2)	X	X	X	Dose 1 Day 1 until last visit date or the date of discontinuation
	• Duration of transfusion independence	RBC transfusions (refer to Section 9.6.2)	X	X	X	Dose 1 Day 1 until last visit date or the date of discontinuation
	• Time to erythroid response	RBC transfusions (refer to Section 9.6.3.9)	X	X	X	Dose 1 Day 1 through last visit in the double-blind Treatment Period
	• Post-baseline transfusion events frequency versus placebo	RBC transfusions	X	X	X	Dose 1 Day 1 through 3 weeks post last dose

Table 2: Study Endpoints (Continued)

Endpoint	Name	Description	Time of Endpoint Measurement			Timeframe
			24 weeks	48 weeks	Long -Term	
Other Efficacy Endpoints	CCI	CCI Bayesian estimates of subject-specific PK exposure measures (AUC, C_{max}, or other exposure metrics of interest) CCI	X	X	X	Dose 1 Day 1 through up to unblinding.
CCI						

ADA = antidrug (ACE-536) antibody; AE = adverse event; AUC = area under the curve, BSC= best supportive care; Cmax = maximum serum concentration; dw = dry weight; DXA = dual energy x-ray absorptiometry; CCI; HRU = Healthcare resource utilization; ICT = iron chelation therapy; IP = investigational product; LIC = liver iron concentration; MRI = magnetic resonance imaging; NCI CTCAE = National Cancer Institute Common Terminology for Adverse Events; QoL = quality of life; RBC = red blood cell.

3. OVERALL STUDY DESIGN

3.1. Study Design

This is a Phase 3, double-blind, randomized, placebo-controlled, multicenter study to determine the efficacy and safety of luspatercept (ACE-536) plus BSC versus placebo plus BSC in adults who require regular red blood cell transfusion due to β-thalassemia. The study is divided into the Screening/Run-in Period, double-blind Treatment Period, double-blind Long-term Treatment Period, Open-label Phase and Post-treatment Follow-up Period. The overall study design is described in Figure 4. Further clarifications are listed in the subject management decision tree provided in Figure 5.

It is planned to randomize approximately 300 subjects at a 2:1 ratio of luspatercept plus BSC versus placebo plus BSC.

3.1.1. Screening/Run-in Period

Upon giving written informed consent, the subject enters the Screening/Run-in Period to determine eligibility. Subjects' identification (ID) number will be allocated via Interactive Response Technology (IRT) system as detailed in Section 7.3. The Screening/Run-in period will last at least 12 weeks. During the Screening/Run-in Period, the subject will undergo safety and other assessments to determine eligibility for the study as detailed in Table 3, Table of Events. Subject eligibility will be reviewed with the Sponsor, prior to the subject's randomization via the IRT. Re-screening is allowed and a new subject ID number will be assigned.

The regularly transfused β-thalassemia subjects must have at least 24 weeks of documented transfusion history available (including Hb levels prior to each transfusion, the number of units transfused, the date of transfusion) prior to randomization. Twelve weeks of transfusion history will be collected prospectively during the Screening/Run-in Period, in addition to the 12 weeks historical retrospective data that will be further recorded in the subject's electronic case report form (eCRF).

The 24-week transfusion history prior to randomization into the Treatment Period will be used to determine the baseline RBC transfusion burden as well as the mean pre-transfusion Hb value for each study subject.

Subjects will be stratified prior to randomization based on:

1. Geographical region[a] (Viprakasit, 2013):
   - North America and Europe
   - Middle East and North Africa
   - Asia-Pacific

[a] See Appendix E for list of countries within each region.

3.1.2. Treatment Period

The subject will enter the Treatment Period once the subject has fulfilled the required assessments in the Screening Period, has fulfilled the eligibility criteria and has been randomized

via the IRT system as detailed in Section 7.3. Eligible subjects will be randomized at a ratio of 2:1 to luspatercept plus BSC or placebo plus BSC treatment at a starting dose level of 1 mg/kg. The maximum total dose per administration should not exceed 120 mg. After the subject randomization via the IRT system the first dose of IP (Dose 1 Day 1) should be administered within 3 days of randomization and can be on the same day of randomization, provided that the eligibility criteria are met. If a transfusion occurs on Dose 1 Day 1, the first dose of luspatercept should not be given within 1 hour before the start of the transfusion, or 2 hours after the end of the transfusion.

Best supportive care will be available to subjects in both the luspatercept and placebo groups. This will include RBC transfusions, iron-chelating agents, use of antibiotic therapy, antiviral and antifungal therapy, and/or nutritional support as needed, thus minimizing the safety risk to subjects. Please refer to Section 8.1.3 for concomitant iron chelation therapy use and Section 8.1.4 for concomitant RBC transfusions.

The double-blind Treatment Period is considered the first 48 weeks starting from Study Day 1 (ie, Dose 1 Day 1), independent of dose delays. Treatment with investigational product for each subject begins on Study Day 1. Subjects will begin treatment at a starting dose level of 1 mg/kg administered by subcutaneous (SC) injection once every 3 weeks for 48 weeks. The starting dose with dose modification is detailed in Section 7. Study visits and serial measurements of safety and efficacy will be performed as described in Table 3, Table of Events.

The dose level for subjects can be titrated (increased) stepwise up to a maximum of 1.25 mg/kg during the Treatment Period as well as during the Long-term Treatment Period and Open-label Phase unless dose modification is required. Dose titration will be based on erythroid response during the previous two dose cycles (~6 weeks). Dose titration rules are further defined in Table 4, and Section 7.2.1.1. Subject's dose titration should be reviewed with the Sponsor and performed in the IRT.

The dose of luspatercept or placebo for each subject can be delayed and/or reduced as per the dose modification, dose reduction and dose delay guidelines as detailed in Table 5. Subjects will be discontinued from study treatment as appropriate, for reasons listed in Section 11.

The decision to discontinue a subject from study treatment remains the responsibility of the treating physician; the Sponsor will not delay or refuse it. However, prior to a decision of discontinuing a subject, it is recommended that the Investigator contact the medical monitor and forward appropriate supporting documents for review and discussion.

3.1.3. Long-term Treatment Period

At the investigator's discretion, subjects completing the 48-week double-blind Treatment Period will be able to continue receiving the IP to which they were initially randomized (ie, luspatercept or placebo) in a double-blind Long-term Treatment Period for up to 48 weeks after the first dose of the last subject or until the date of unblinding.

The double-blind Long-term Treatment Period will continue until all subjects have either completed 48 weeks of the double-blind Treatment Period or discontinued before reaching 48 weeks of the double-blind Treatment Period, or in the event the study is unblinded per Data Monitoring Committee (DMC) recommendation. Treatment during the Long-term Treatment

Period is subject to dose titration, dose delay and dose reduction, and treatment discontinuation as described in Section 7, Table 4 and Table 5.

Subject's dose titration should be reviewed with the Sponsor and performed in the IVRS.

Dose titration during the Long-term Treatment Period is allowed. Dose titration rules are defined as described in Table 4 and Section 7.2.1.1. Please refer to Section 8.1.3 for concomitant iron chelation therapy use and Section 8.1.4 for concomitant RBC transfusions.

Subjects will be discontinued from study as appropriate, for reasons listed in Section 11.

Subjects who continue to receive treatment with luspatercept or placebo when the study is unblinded (approximately 48 weeks after the first dose of the last subject) may opt to continue receiving luspatercept in the Open-label Phase or to discontinue treatment and enter the Post-treatment Follow-up Period.

3.1.4. Open-label Phase

Open-label Phase will begin after the study unblinding. The commencement of this Open-label Phase will be determined by the enrollment rate and by the availability of primary analysis data that justify the use of luspatercept in an Open-label Phase, which will be reviewed by the independent external DMC. After DMC review of safety and efficacy, DMC will determine if the use of luspatercept in subjects previously randomized to receive placebo in this Open-label phase is safe and recommended, and if subjects already on luspatercept can continue to be treated at their current dose level (best supportive care is allowed). In the Open-label Phase, subjects may receive luspatercept until all subjects initially assigned to luspatercept in the double-blind Treatment Period, complete the total treatment duration of 5 years from subjects' Dose 1 Day 1 in the treatment period of this ACE-536-B-THAL-001 study or discontinue early.

Access to the Open-label Phase:

Subjects initially assigned to luspatercept:

- should not have discontinued the double-blind phase, in order to be eligible for the Open-label Phase.
- will be in the Open-label Phase until all subjects on luspatercept complete the total treatment duration of 5 years from subjects' Dose 1 Day 1 in the treatment period of this ACE-536-B-THAL-001 study or discontinue earlier.

Subjects initially assigned to placebo:

- can enter the Open-label Phase only if the DMC allows it, as outlined above.
- can enter the Open-label Phase even if they have discontinued the double-blind phase, but were compliant with the protocol 48 weeks post Dose 1 Day 1, and continue their participation in the Post-treatment Follow-up Period until the time of unblinding.can stay in the Open-label Phase until all subjects initially assigned to luspatercept complete the total treatment duration of 5 years from subjects' Dose 1 Day 1 in the treatment period of this ACE-536-B-THAL-001 study or discontinue earlier.
- at the time of unblinding, placebo subjects who discontinued treatment and continued their participation in the Post-treatment Follow-up Period until the time of unblinding

will have the opportunity to enter the Open-label Phase, if they still fulfill the following main eligibility criteria, prior to receiving their first dose of luspatercept:

- Inclusion criteria: numbers 6 and 8 (Refer to Section 4.2);
- Exclusion criteria: numbers "1-10, 15-17 and 21" (Refer to Section 4.3)

Per Investigator's request, subjects who discontinue luspatercept in the double-blind Treatment Period for reasons not related to subject's safety, and are still in the Post-treatment Follow-up Period at the time of unblinding, may access the Open-label Phase and be re-treated with luspatercept after consultation with Sponsor Medical Monitor and review of safety and efficacy data as long as they still fulfill the above eligibility criteria prior to Dose 1 Day 1.

Note: Subjects who discontinue from the study before the unblinding and without completing the PTFP period are not allowed to re-enter this study and access luspatercept in the Open-label Phase.

Subjects will be discontinued from study as appropriate, for reasons listed in Section 11.

3.1.5. Post-treatment Follow-up Period

All subjects who discontinue treatment will undergo a 156-week Post-treatment Follow-up Period, following the last dose of IP.

All subjects who discontinue treatment after completion of at least 48 weeks of the double-blind Treatment Period will enter the Post-treatment Follow-up Period for 156 weeks (ie 3 years). Subjects who enter the Long-term Treatment Period or Open-label Phase and discontinue treatment during those periods, will also enter the Post-treatment Follow-up Period for 156 weeks. The Post-treatment Follow-up Period will begin after the last IP dose is received, as per the visit schedule laid down in protocol Table 3 (ie, 9-week follow-up, Week 24, and every 24 weeks after the last dose up to Week 144, follow-up by End of Study visit at Week 156).

Early discontinued subjects, ie, subjects who discontinue before completing 48 weeks in the double-blind Treatment Period, will continue to be monitored on Week 9, followed by Week 24, Week 48, Week 72, Week 96, Week 120, Week 144 after the last dose up to Week 156 (assessments defined in Table 3, Table of Events).

When the study is unblinded: (i) subjects on luspatercept stay in the Post-treatment Follow-up Period for up to 156 weeks; (ii) subjects on placebo will stay up to unblinding and will be moved to the Open-label Phase after fulfilling appropriate eligibility criteria (refer to Section 3.1.4).

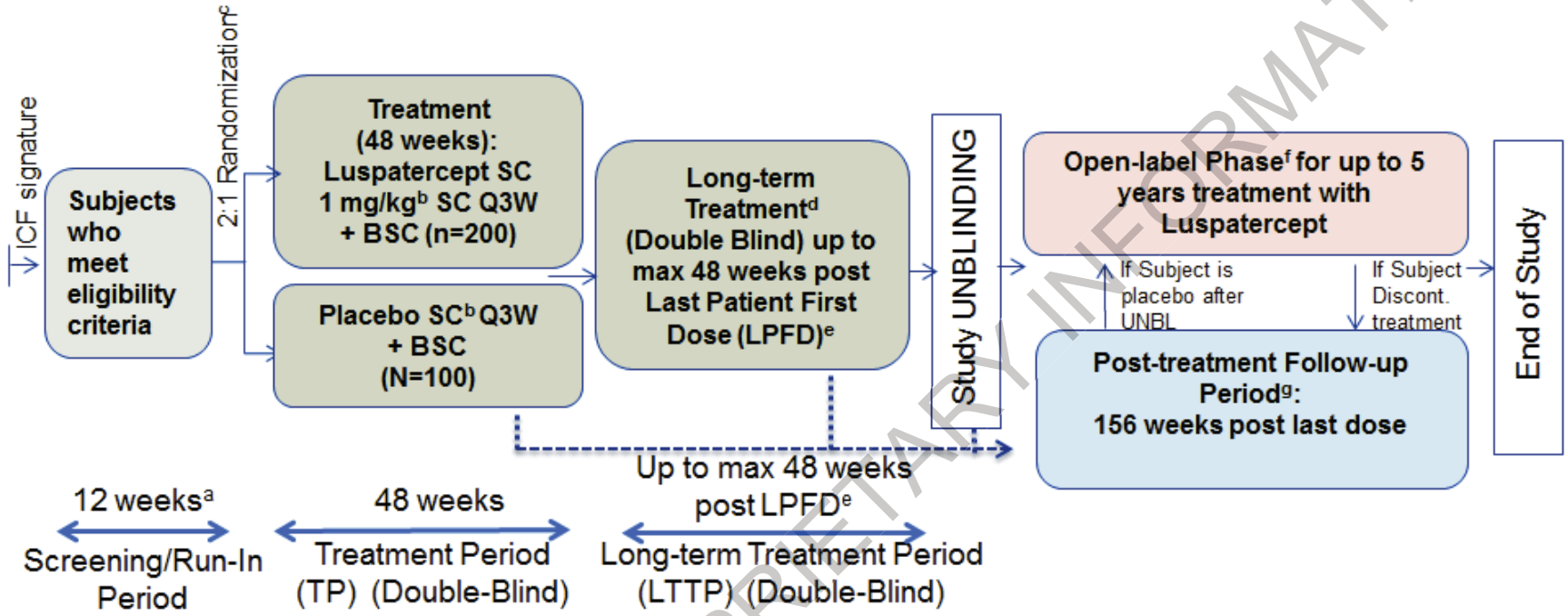
Refer to the subject management decision tree detailed in Figure 5.

3.1.6. Committees

The conduct of this study will be overseen by an independent external DMC and by a Steering Committee. The DMC will review unblinded data, while the Steering Committee will not have access to unblinded data. Refer to Section 9.10.2 and Section 9.10.3 for additional information.

The study will be conducted in compliance with the International Council on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use/Good Clinical Practice (GCP) and applicable regulatory requirements.

Figure 4: Overall Study Design



BSC = best supportive care; DMC = Data Monitoring Committee; ICF = informed consent form; Q3W = every 3 weeks; SC = subcutaneous; UNBL = unblinding; LPFD = last patient first dose.

[a] The historical documentation of transfusion dependence for β-thalassemia subjects (including units transfused and hemoglobin (Hb) levels measured prior to each transfusion) for 24 weeks prior to subject randomization, should be available.

[b] Dose may be titrated up to a maximum of 1.25 mg/kg.

[c] Randomization will be 2:1, luspatercept plus BSC versus placebo plus BSC.

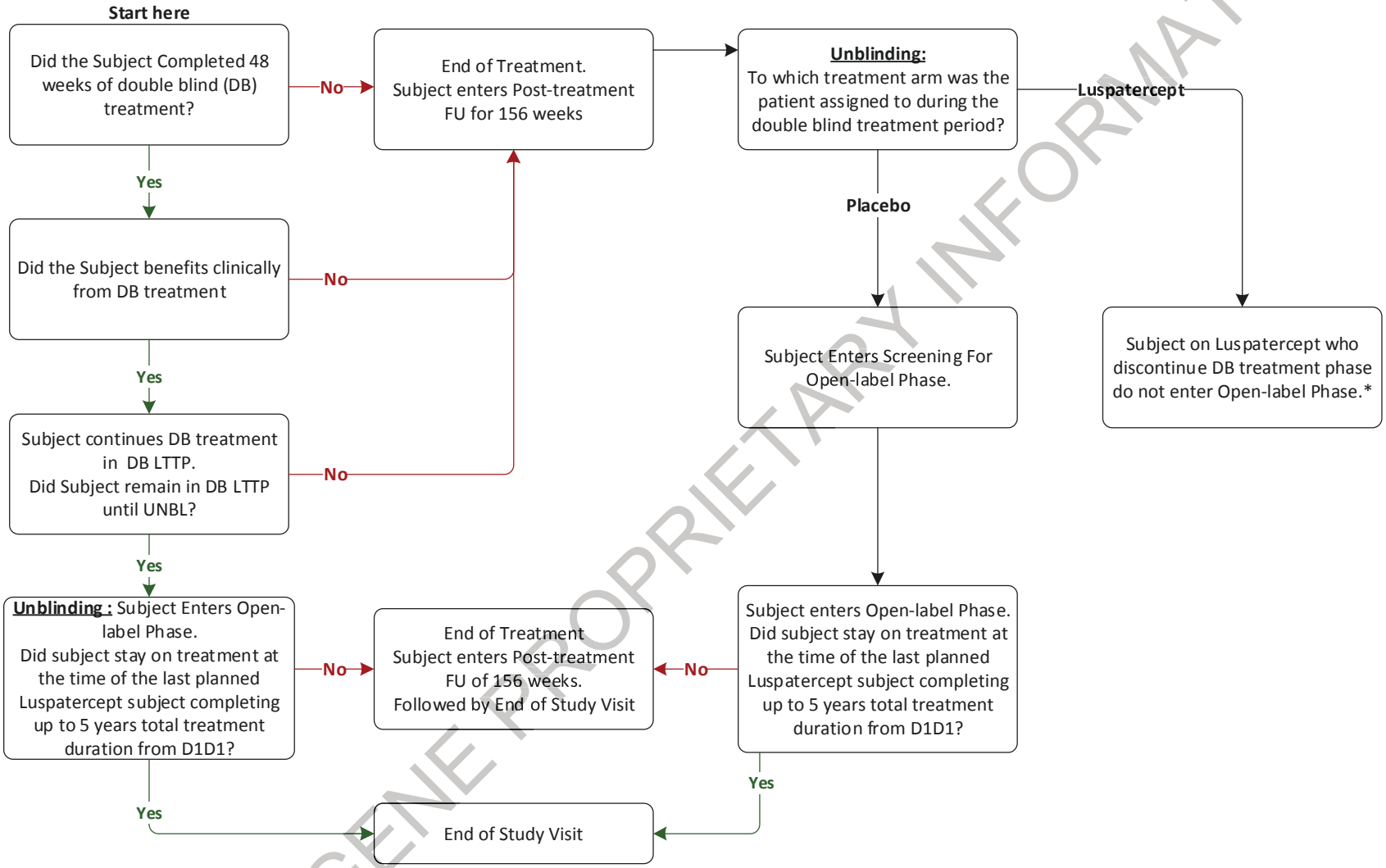
[d] All subjects, who complete 48 weeks of the double-blind Treatment Period of this study will have the opportunity to continue to a double-blind Long-Term Treatment Period at the Investigator's discretion. Subjects who do not enroll in the double-blind Long-Term Treatment Period or who discontinue early will proceed to the Post-treatment Follow-up Period.

[e] Maximum duration of 48 weeks post LPFD, or when all subjects completed 48 weeks of double-blind treatment or discontinued before reaching 48 weeks double-blind treatment, or in the event the study is unblinded per DMC recommendation.

[f] Open-label Phase: Subjects who were compliant with the protocol 48 weeks post Dose 1 Day 1 can enter in the Open-label Phase, unless medically contraindicated and as described in Section 3.1.4.

[g] Early discontinued subjects, ie, subjects who discontinue before completing the double-blind treatment period (48 weeks), will continue to be monitored on week 9, followed by 24, 48, 72, 120, 144 after the last dose up to Week 156, ie 3 years (refer to Section 3.1.5).

Figure 5: Subject Management Decision Tree



DB = double-blind; LTTP = Long-term Treatment Period; UNBL = unblinding; D1D1 = Dose 1 Day 1; FU = Follow-up.
*refer also to Section 3.1.4.

3.2. Study Duration for Subjects

Study participation for each subject includes a Screening/Run-in Period of at least 12 weeks, a 48-week placebo-controlled double-blind Treatment Period, followed by a double-blind Long-term Treatment Period and an Open-label Phase. The Post-treatment Follow-up Period will last 156 weeks (ie 3 years) post last dose.

The study will be comprised of the following periods:

Screening/Run-in Period, will be at least 12 weeks prior to randomization.

Treatment Period, which will be approximately 48 weeks, independent of Dose Delays beginning with Dose 1 Day 1.

Long-term Treatment Period, the double-blind Long-term Treatment Period will continue until all subjects complete 48 weeks of double-blind treatment or discontinue before reaching 48 weeks of the double-blind Treatment Period, or at the time the study is unblinded (per DMC recommendation), whichever occurs first.

Open-label Phase, the Open-label Phase will continue until all subjects initially assigned to luspatercept in the double-blind Treatment Period, complete the total treatment duration of 5 years from subjects' Dose 1 Day 1 in the treatment period of this ACE-536-B-THAL-001 study or discontinue earlier.

Post-treatment Follow-Up Period, all subjects who discontinue treatment will undergo a 156-week (ie 3 years) Post-treatment Follow-up Period, following the last dose of IP. Additional information on the visits to complete when subjects discontinue treatment is presented in Section 3.1.5.

End of Treatment for each individual subject is defined as the date of the last visit in the Treatment Period, the Long-term Treatment Period, or in the Open-label Phase, whichever is the later date.

End of Study for each individual subject occurs at the time of completion of the 156-week Post-treatment Follow-Up Period or at the time of the end of the Open-label Phase (if subjects are still on treatment, see the description above) or at the time of End of Trial as defined below.

3.3. End of Trial

The End of Trial is defined as when all subjects initially assigned to luspatercept in the double-blind Treatment Period, reach the maximum treatment duration of 5 years from subjects' Dose 1 Day 1 in the treatment period of this ACE-536-B-THAL-001 study or discontinue earlier and complete the 156 weeks of the Post-treatment Follow-up Period, whichever occurs later; or the date of receipt of the last data point from the last subject that is required for primary, secondary, CCI analysis, as pre-specified in the protocol and/or Statistical Analysis Plan (SAP), whichever is the later date.

The Sponsor may end the trial when all key endpoints and objectives of the study have been analyzed, and the availability of a roll-over/extension protocol exists into which any subjects who remain on study may be consented and continue to receive access to luspatercept. Such a protocol would be written for a compound that would not yet be commercially available.

4. STUDY POPULATION

4.1. Number of Subjects

Approximately 300 subjects diagnosed with transfusion-dependent β-thalassemia (including Hemoglobin E/β-thalassemia, excluding Hemoglobin S/β-thalassemia and Hemoglobin H) requiring regular transfusions will be randomized worldwide.

4.2. Inclusion Criteria

Subjects must satisfy the following criteria to be enrolled in the study:

1. Male or female, ≥ 18 years of age at the time of signing the informed consent document (ICF).
2. Subject must understand and voluntarily sign an ICF prior to any study-related assessments/procedures being conducted.
3. Subject is willing and able to adhere to the study visit schedule (eg, not scheduled to receive HSCT) and other protocol requirements.
4. Documented diagnosis of β-thalassemia or Hemoglobin E/β-thalassemia (β-thalassemia with mutation and/or multiplication of alpha globin is allowed).
5. Regularly transfused, defined as: 6-20 RBC units* in the 24 weeks prior to randomization and no transfusion-free period for > 35 days during that period.

* Sites who prescribe transfusions and have the transfusion records only in volumes should use for conversion of volume to units the below criteria, in order to obtain number of units within the last 24 weeks to assess the eligibility: 1 unit in this protocol refers to a quantity of packed RBCs approximately 200-350 mL. (i) sites who use transfusion bags within this range, or ≥ 350 mL, the conversion in units should be done by dividing the volume transfused to the patient by 350 mL, (ii) sites who use transfusion bags < 200 mL, the conversion in units should be done by dividing the volume transfused to the patient by 200 mL.

6. Performance status: Eastern Cooperative Oncology Group (ECOG) score of 0 or 1.
7. A female of childbearing potential (FCBP) for this study is defined as a female who: 1) has achieved menarche at some point, 2) has not undergone a hysterectomy or bilateral oophorectomy or 3) has not been naturally postmenopausal (amenorrhea following cancer therapy does not rule out childbearing potential) for at least 24 consecutive months (ie, has had menses at any time in the preceding 24 consecutive months). FCBP participating in the study must:
    a. Have two negative pregnancy tests as verified by the Investigator prior to starting study therapy. She must agree to ongoing pregnancy testing during the course of the study, and after end of study treatment. This applies even if the subject practices true abstinence** from heterosexual contact.
    b. Either commit to true abstinence** from heterosexual contact (which must be reviewed on a monthly basis and source documented). If a FCBP engages in sexual activity that may result in a pregnancy, she must agree to use, and be able to comply with, effective*** contraception without interruption, 28 days prior to starting investigational product, during the study therapy (including dose interruptions), and

for 12 weeks [redacted] after discontinuation of study therapy.

8. Male subjects must:

   a. Practice true abstinence** (which must be reviewed on a monthly basis) or agree to use a condom during sexual contact with a pregnant female or a female of childbearing potential while participating in the study, during dose interruptions and for at least 12 weeks [redacted] following investigational product discontinuation, even if he has undergone a successful vasectomy.

** True abstinence is acceptable when this is in line with the preferred and usual lifestyle of the subject. [Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.]

*** Agreement to use highly effective methods of contraception that alone or in combination result in a failure rate of a Pearl index of less than 1% per year when used consistently and correctly throughout the course of the study. Such methods include: Combined (estrogen and progesterone/progestin containing) hormonal contraception: Oral; Intravaginal; Transdermal; Progestogen/progestin only hormonal contraception associated with inhibition of ovulation: Oral; Injectable hormonal contraception; Implantable hormonal contraception; Placement of an intrauterine device (IUD); Placement of an intrauterine hormone-releasing system (IUS); Bilateral tubal occlusion; Vasectomized partner; Sexual Abstinence.

4.3. Exclusion Criteria

The presence of any of the following will exclude a subject from enrollment:

1. Any significant medical condition, laboratory abnormality, or psychiatric illness that would prevent the subject from participating in the study.
2. Any condition including the presence of laboratory abnormalities, which places the subject at unacceptable risk if he/she were to participate in the study.
3. Any condition that confounds the ability to interpret data from the study.
4. A diagnosis of Hemoglobin S/β-thalassemia or alpha (α)-thalassemia (eg, Hemoglobin H).
5. Evidence of active hepatitis C (HCV) infection as demonstrated by a positive HCV-RNA test of sufficient sensitivity, or active infectious hepatitis B as demonstrated by the presence of HBsAg and/or HBVDNA-positive, or known positive human immunodeficiency virus (HIV).

Note: Subjects receiving antiviral therapies should have 2 negative HCVRNA tests 3 months apart.(ie, one test at the end of the antiviral therapy and a second test 3 months following the first test).

6. DVT or stroke requiring medical intervention ≤ 24 weeks prior to randomization.
7. Use of chronic anticoagulant therapy is excluded, unless the treatment stopped at least 28 days prior to randomization. Anticoagulant therapies used for prophylaxis for surgery or high risk procedures as well as low-molecular-weight (LMW) heparin for superficial venous thrombosis and chronic aspirin are allowed.
8. Platelet count > 1000 x 10^9/L.

9. Poorly controlled diabetes mellitus within 24 weeks prior to randomization as defined by short term (eg, hyperosmolar or ketoacidotic crisis) and/or history of diabetic cardiovascular complications (eg, stroke or myocardial infarction).
10. Treatment with another investigational drug or device ≤ 28 days prior to randomization.
11. Prior exposure to sotatercept (ACE-011) or luspatercept (ACE-536).
12. Use of an erythropoiesis-stimulating agent (ESA) ≤ 24 weeks prior to randomization.
13. Iron chelation therapy, if initiated ≤ 24 weeks prior to randomization (allowed if initiated > 24 weeks before or during treatment).
14. Hydroxyurea treatment ≤ 24 weeks prior to randomization.
15. Pregnant or lactating females.
16. Uncontrolled hypertension. Controlled hypertension for this protocol is considered ≤ Grade 1 according to NCI CTCAE version 4.0 (current active minor version).
17. Major organ damage, including:
    a. Liver disease with alanine aminotransferase (ALT) > 3 x the upper limit of normal (ULN) or history of evidence of cirrhosis.
    b. Heart disease, heart failure as classified by the New York Heart Association (NYHA) classification 3 or higher, or significant arrhythmia requiring treatment, or recent myocardial infarction within 6 months of randomization.
    c. Lung disease, including pulmonary fibrosis or pulmonary hypertension which are clinically significant ie, ≥ Grade 3 NCI CTCAE version 4.0 (current active minor version).
    d. Creatinine clearance < 60 mL/min (per Cockroft-Gault formula).
18. Proteinuria ≥ Grade 3 according to NCI CTCAE version 4.0 (current active minor version).
19. Chronic systemic glucocorticoids ≤ 12 weeks prior to randomization (physiologic replacement therapy for adrenal insufficiency is allowed). Single day glucocorticoid treatment (eg, for prevention or treatment of transfusion reactions, is allowed).
20. Major surgery ≤ 12 weeks prior to randomization (subjects must have completely recovered from any previous surgery prior to randomization).
21. History of severe allergic or anaphylactic reactions or hypersensitivity to recombinant proteins or excipients in the investigational product (see Investigator Brochure).
22. Cytotoxic agents, immunosuppressants ≤ 28 days prior to randomization (ie, anti-thymocite globulin (ATG) or cyclosporine)
23. History of malignancy with the exception of:
    a. Curatively resected nonmelanoma skin cancer.
    b. Curatively treated cervical carcinoma in situ.
    c. Other solid tumor with no known active disease in the opinion of the investigator.

5. TABLE OF EVENTS

Table 3: Table of Events

Assessments	Section	Screening/ Run-in Period Week -12 to Day -1	Treatment Period (double-blinded)				Long-term Treatment Period[13] (double-blinded) every 3-wk visits from Week 49 (ie, Dose 17 if no dose delays) /Day 1 (± 5 days)	Open-label Phase, every 3-wk visits from unblinding (± 5 days)		Day 22 Post Last Dose / Treatment D/C[7]	Post-treatment Follow-up Period (± 7 days)			
			Luspatercept/ placebo Dose 1 Schedule (+ 7 days)	Luspatercept/placebo Doses 2 up to max 48 weeks Treatment (± 3 days)										
			Day 1	Day 1	Day 8	Day 15		Dose 1 for Placebo, Discont.[18]	Dose X Day 1[19]		Follow-up Week 9 (from last dose)[23]	Only if[14] Early Discont. – Follow-up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	All subjects: Follow-Up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	End of Study[1][5]/ Follow-up week 156 (from last dose)[2][3]
Study Entry and General Assessments														
Informed Consent	6.1	X	-	-	-	-	-	-	-	-	-	-	-	-
Inclusion/Exclusion Criteria	6.1	X	-	-	-	-	-	X	-	-	-	-	-	-
Demographics	6.1	X	-	-	-	-	-	-	-	-	-	-	-	-
Medical History	6.1	X	-	-	-	-	-	-	-	-	-	-	-	-
β-thalassemia genotype (ie, beta and alpha globin mutations, only if not available in subject medical history)[20]	6.1	X	-	-	-	-	-	-	-	-	-	-	-	-
Hepatitis B & C[16]	6.1	X	-	-	-	-	-	-	-	-	-	-	-	-
Iron Chelation Therapy	6.1 to 6.6	X	Record on ongoing basis, until 9 weeks Post-last Dose[24]											
Other Prior / Concomitant / Post (disease specific) Medications / Therapies	6.1 to 6.6	X	Record on ongoing basis, until 9 weeks Post-last Dose[24]											

Table 3: Table of Events (Continued)

Assessments	Section	Screening/ Run-in Period Week -12 to Day -1	Treatment Period (double-blinded)				Long-term Treatment Period[13] (double-blinded) every 3-wk visits from Week 49 (ie, Dose 17 if no dose delays) /Day 1 (± 5 days)	Open-label Phase, every 3-wk visits from unblinding (± 5 days)		Day 22 Post Last Dose / Treatment D/C[7]	Post-treatment Follow-up Period (± 7 days)			
			Luspatercept/ placebo Dose 1 Schedule (+7 days)	Luspatercept/placebo Doses 2 up to max 48 weeks Treatment (± 3 days)							Follow-up Week 9 (from last dose)[23]	Only if[14] Early Discont. – Follow-up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	All subjects: Follow-Up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	End of Study[15] / Follow-up week 156 (from last dose)[23]
			Day 1	Day 1	Day 8	Day 15		Dose 1 for Placebo, Discont.[18]	Dose X Day 1[19]					
Prior/concomitant/post procedures (eg, surgery, radiation therapy)	6.1 to 6.6	X	Record on ongoing basis, until 9 weeks Post-last Dose[24]											
Transfusion Assessment (≥ 24 weeks of history prior to Dose 1 Day 1)	6.1 to 6.6	X	Record on ongoing basis, until 9 weeks Post-last Dose[24]											
Subject Transfusion – information collection[25]	6.1 to 6.6	X	Record on ongoing basis, until 9 weeks Post-last Dose[24]											
Safety Assessments														
Adverse Events	6.1 to 6.7	Continuous starting after informed consent signature, until 9 weeks post last dose, related AE to be reported until End of Study												
Malignancy and Pre-malignancy Reporting (Section 10.5.2)[26]	6.1 to 6.7	Continuous starting after informed consent signature, regardless of causality reporting occurrence of any case										X[26]	X[26]	X[26]
Vital Signs[1]	6.1 to 6.7	X (within 4 wks prior Dose 1 Day 1)	X	X	-	-	X	X	X	X	X			

Table 3: Table of Events (Continued)

Assessments	Section	Screening/ Run-in Period Week -12 to Day -1	Treatment Period (double-blinded)				Long-term Treatment Period[13] (double-blinded) every 3-wk visits from Week 49 (ie, Dose 17 if no dose delays) /Day 1 (± 5 days)	Open-label Phase, every 3-wk visits from unblinding (± 5 days)		Day 22 Post Last Dose / Treatment D/C[7]	Post-treatment Follow-up Period (± 7 days)			
			Luspatercept/ placebo Dose 1 Schedule (+7 days)	Luspatercept/placebo Doses 2 up to max 48 weeks Treatment (± 3 days)							Follow-up Week 9 (from last dose) [23]	Only if [14] Early Discont. – Follow-up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	All subjects: Follow-Up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	End of Study[15] / Follow-up week 156 (from last dose)[23]
			Day 1	Day 1	Day 8	Day 15		Dose 1 for Placebo, Discont.[18]	Dose X Day 1[19]					
Height (at Screening only)/Weight	6.1 to 6.7	X (within 4 wks prior Dose 1 Day 1)	X	X	-	-	X	X	X	X	X			
ECOG Performance Status	6.1 and 6.4	X (within 4 wks prior Dose 1 Day 1)	X	-	-	-	-	X	-	-	-	-	-	-
12-Lead Electrocardiogram (ECG) – read locally	6.1, 6.2, 6.4, and 6.5	X (within 4 wks prior Dose 1 Day 1)	-	-	X (Dose 6 only)	-	-	X	-	X	-	-	-	-
Cardiac Doppler Echocardiography, MUGA or MRI[17; 21]: LVEF	6.1, 6.2, 6.3	X	-	X (wk 24, wk 48 only)-	-	-	X (wk 96 only) [9]	-	-	-	-	-	-	-
Pregnancy Testing[2]	6.1 to 6.6	X (within 4 wks prior Dose 1 Day 1; serum only)	X	X	-	-	X	X	X	X	X	-	-	-
Menstrual status (female only)	6.1 to 6.6	X	X	X	-	-	X	X	X	X	X	-	-	-

Table 3: Table of Events (Continued)

Assessments	Section	Screening/ Run-in Period Week -12 to Day -1	Treatment Period (double-blinded)				Long-term Treatment Period[13] (double-blinded) every 3-wk visits from Week 49 (ie, Dose 17 if no dose delays) /Day 1 (± 5 days)	Open-label Phase, every 3-wk visits from unblinding (± 5 days)		Day 22 Post Last Dose / Treatment D/C[7]	Post-treatment Follow-up Period (± 7 days)			
			Luspatercept/ placebo Dose 1 Schedule (+7 days)	Luspatercept/placebo Doses 2 up to max 48 weeks Treatment (± 3 days)							Follow-up Week 9 (from last dose)[23]	Only if[14] Early Discont. – Follow-up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	All subjects: Follow-Up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	End of Study[15] / Follow-up week 156 (from last dose)[23]
			Day 1	Day 1	Day 8	Day 15		Dose 1 for Placebo, Discont.[18]	Dose X Day 1[19]					
Hematology[3] (central lab; use local lab for predose hemoglobin, in case of AE, or between doses; reticulocytes and erythroblast to be measured by local lab; in Open-label Phase – use local labs)	6.1 to 6.6	X (within 4 wks prior Dose 1 Day 1)	X	X	X (Dose 6 only)	X (Dose 6 only)	X[11]	X	X	X	X	-	-	-
Serum Chemistry[4] (predose: central lab; use local lab in case of AE, or between doses; in Open-label Phase – use local labs)	6.1 to 6.6	X (within 4 wks prior Dose 1 Day 1)	X	X	-	-	X[11] (every 4 doses)	X	-	X	X	-	-	-
Urinalysis: microalbumin, creatinine, microalbumin/creatinine ratio (morning void; central lab; in Open-label Phase – use local labs)	6.1.1 to 6.6	X (within 4 wks prior Dose 1 Day 1)	-	X (every 4 doses)	-	-	X[11] (every 4 doses)	X	-	X	X	-	-	-

Table 3: Table of Events (Continued)

Assessments	Section	Screening/ Run-in Period Week -12 to Day -1	Treatment Period (double-blinded)				Long-term Treatment Period[13] (double-blinded) every 3-wk visits from Week 49 (ie, Dose 17 if no dose delays) /Day 1 (± 5 days)	Open-label Phase, every 3-wk visits from unblinding (± 5 days)		Day 22 Post Last Dose / Treatment D/C[7]	Post-treatment Follow-up Period (± 7 days)			
			Luspatercept/ placebo Dose 1 Schedule (+7 days)	Luspatercept/placebo Doses 2 up to max 48 weeks Treatment (± 3 days)							Follow-up Week 9 (from last dose)[23]	Only if[14] Early Discont. – Follow-up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	All subjects: Follow-Up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	End of Study[15] / Follow-up week 156 (from last dose)[23]
			Day 1	Day 1	Day 8	Day 15		Dose 1 for Placebo, Discont.[18]	Dose X Day 1[19]					
Serum Erythropoietin (predose; central lab)	6.1.1 to 6.5	-	X	X (every 4 doses)	-	-	-	-	-	X	-	-	-	-
Serum PK (predose if on dosing day; central lab)[10]	6.8	-	X	X (Dose 2, 3, 4, 5 and 6, 8, 10, 12, 14, 16)	X (Dose 6 only)	X (Dose 6 only)	X (every 6 doses, eg, at Dose 22, 28, 34….)	-	-	-	-	-	-	-

Table 3: Table of Events (Continued)

Assessments	Section	Screening/ Run-in Period Week -12 to Day -1	Treatment Period (double-blinded)				Long-term Treatment Period[13] (double-blinded) every 3-wk visits from Week 49 (ie, Dose 17 if no dose delays) /Day 1 (± 5 days)	Open-label Phase, every 3-wk visits from unblinding (± 5 days)		Day 22 Post Last Dose / Treatment D/C[7]	Post-treatment Follow-up Period (± 7 days)			
			Luspatercept/ placebo Dose 1 Schedule (+7 days)	Luspatercept/placebo Doses 2 up to max 48 weeks Treatment (± 3 days)							Follow-up Week 9 (from last dose)[23]	Only if[14] Early Discont. – Follow-up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	All subjects: Follow-Up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	End of Study[15] / Follow-up week 156 (from last dose)[23]
			Day 1	Day 1	Day 8	Day 15		Dose 1 for Placebo, Discont.[18]	Dose X Day 1[19]					
Antidrug antibody (ADA)[5, 10] (central lab)	6.9	-	X	X (Dose 2, 4 and 6; 8, 12, 16)	-	-	X (every 6 doses, eg, at Dose 22, 28, 34….)[10]	X (only if positive at unblinding, every 6 doses up to 2 years of the Dose 1 Day 1 of the DBTP)	X (only if positive at unblinding, every 6 doses up to 2 years of Dose 1 Day 1 of the DBTP)[10]	-	X	X (every 24 wks as applicable)[10]	X (every 24 wks as applicable)[10]	-

Table 3: Table of Events (Continued)

Assessments	Section	Screening/ Run-in Period Week -12 to Day -1	Treatment Period (double-blinded)				Long-term Treatment Period[13] (double-blinded) every 3-wk visits from Week 49 (ie, Dose 17 if no dose delays) /Day 1 (± 5 days)	Open-label Phase, every 3-wk visits from unblinding (± 5 days)		Day 22 Post Last Dose / Treatment D/C[7]	Post-treatment Follow-up Period (± 7 days)			
			Luspatercept/ placebo Dose 1 Schedule (+7 days)	Luspatercept/placebo Doses 2 up to max 48 weeks Treatment (± 3 days)							Follow-up Week 9 (from last dose) [23]	Only if [14] Early Discont. – Follow-up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	All subjects: Follow-Up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	End of Study[1] [5]/ Follow-up week 156 (from last dose)[2] [3]
			Day 1	Day 1	Day 8	Day 15		Dose 1 for Placebo, Discont.[18]	Dose X Day 1[19]					
Efficacy and Other Assessments														
MRI for LIC (with T2* or R2, mg/g dw) [12, 17]	6.1, 6.1.1, 6.2, 6.3, 6.4, 6.5	X	-	X (wk 24 & wk 48)	-	-	wk 96 only, if applicable[9]	-	wk 96 only, if applicable[9]	X (if not performed within the last 12 wks)	-	X (wk 48) post Dose 1)	-	-
MRI or abdominal ultrasound for spleen measurements for spleen, unless splenectomized[12,17]	6.1, 6.1.1, 6.2, 6.3, 6.4, 6.5	X	-	X (wk 24 & wk 48)	-	-	wk 96 only, if applicable[9]	-	wk 96 only, if applicable[9]	X (if not performed within the last 12 wks)	-	X (wk 48) post Dose 1)	-	-
MRI for myocardial iron (T2*; ms) [12,17]	6.1, 6.1.1, 6.2, 6.3, 6.5	X	-	X (wk 48)	-	-	wk 96 only, if applicable [9]	-	wk 96 only, if applicable[9]	X (if not performed within the last 12 wks)	-	X (wk 48) post Dose 1)	-	-
DXA scan[6,17] – total hip, lumbar spine (read locally)	6.1, 6.1.1, 6.2, 6.3, 6.4, 6.5	X	-	X (wk 48)	-	-	wk 96 only, if applicable [9]	-	wk 96 only, if applicable[9]	X(if not performed within the last 24 wks)	-	X (wk 48) post Dose 1)	-	-

Table 3: Table of Events (Continued)

Assessments	Section	Screening/ Run-in Period Week -12 to Day -1	Treatment Period (double-blinded)				Long-term Treatment Period[13] (double-blinded) every 3-wk visits from Week 49 (ie, Dose 17 if no dose delays) /Day 1 (± 5 days)	Open-label Phase, every 3-wk visits from unblinding (± 5 days)		Day 22 Post Last Dose / Treatment D/C[7]	Post-treatment Follow-up Period (± 7 days)			
			Luspatercept/ placebo Dose 1 Schedule (+7 days)	Luspatercept/placebo Doses 2 up to max 48 weeks Treatment (± 3 days)							Follow-up Week 9 (from last dose) [23]	Only if [14] Early Discont. – Follow-up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	All subjects: Follow-Up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	End of Study[15] / Follow-up week 156 (from last dose)[23]
			Day 1	Day 1	Day 8	Day 15		Dose 1 for Placebo, Discont.[18]	Dose X Day 1[19]					
QoL Questionnaire (TranQol, SF36), assessments to be performed independent of Dose Delays	6.1, 6.1.1, 6.2, 6.3, 6.11	X (within 4 wks prior Dose 1 Day 1)	-	X (wk 12, 24, 36 & wk 48)	-	-	every 12 weeks	-	-	-	-	-	-	-
Healthcare Resource Utilization	6.1 to 6.6	Record on ongoing basis, until 9 weeks Post-last Dose[24]												
Serum Ferritin (predose, central lab; screening value at least 12 weeks prior to randomization; in Open-label Phase – use local labs)	6.1 to 6.6	X (within 4 wks prior Dose 1 Day 1)	X	X	-	-	X[11] (every 4 doses)	X	-	X	X	-	-	-
CCI [redacted]														

Table 3: Table of Events (Continued)

Assessments	Section	Screening/ Run-in Period Week -12 to Day -1	Treatment Period (double-blinded)				Long-term Treatment Period[13] (double-blinded) every 3-wk visits from Week 49 (ie, Dose 17 if no dose delays) /Day 1 (± 5 days)	Open-label Phase, every 3-wk visits from unblinding (± 5 days)		Day 22 Post Last Dose / Treatment D/C[7]	Post-treatment Follow-up Period (± 7 days)			
			Luspatercept/ placebo Dose 1 Schedule (+7 days)	Luspatercept/placebo Doses 2 up to max 48 weeks Treatment (± 3 days)							Follow-up Week 9 (from last dose) [23]	Only if [14] Early Discont. – Follow-up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	All subjects: Follow-Up Visits weeks 24, 48, 72, 96, 120, 144 (from last dose)[23]	End of Study[15] / Follow-up week 156 (from last dose)[23]
			Day 1	Day 1	Day 8	Day 15		Dose 1 for Placebo, Discont.[18]	Dose X Day 1[19]					
CCI [redacted]														
Investigational Product (IP)														
Administer Luspatercept / Placebo Perform Drug Accountability[8]	6.1.1	-	X	X	-	-	X	X	X	-	-	-	-	-

ADA = antidrug (ACE-536) antibody; AE = adverse event; ALT = alanine aminotransferase; ANC = absolute neutrophil count; AST = aspartate aminotransferase; BUN = blood urea nitrogen; eCRF = electronic case report form; D/C = discontinuation; DBP = diastolic blood pressure; DBTP = double-blind Treatment period; DXA = dual energy x-ray absorptiometry; ECOG = Eastern Cooperative Oncology Group; Early Discont. = early discontinued from treatment subjects; CCI [redacted]; IP= investigational product; Hct = hematocrit; Hb = hemoglobin; HIV = human immunodeficiency virus; ICF = informed consent form; lab = laboratory assessments; LDH = lactic dehydrogenase; LIC= liver iron concentration; LTTP = Long-term Treatment Period; LVEF = left ventricular ejection fraction; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; MRI = magnetic resonance imaging; MUGA = Multi Gated Acquisition Scan; PK = Pharmacokinetic; QoL = quality of life; RBC = red blood cell; RDW = red blood cell distribution width; SAEs = serious adverse events; SBP = systolic blood pressure; SF = Short Form; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase; ToE = Table of Events; WBC = white blood cell.; wk = week; wks = weeks.

[1] Vital signs (including heart rate, seated blood pressure [DBP and SBP], and temperature) will be measured at Screening, on Days 1 of each dose prior to administration of IP, at Treatment Discontinuation, at each visit during post-treatment Follow-Up, and at End of Study. Seated blood pressure should be confirmed by two separate measurements obtained predose and 10 minutes apart. The Investigator should report any clinically significant abnormal findings as adverse events.

[2] Pregnancy test is required for all female subjects of childbearing potential. Serum beta human chorionic gonadotropin (β-hCG pregnancy test (which must be negative) with a minimum sensitivity of 25 mIU/mL will be performed within 4 weeks prior Dose 1 Day 1. Urine (or serum) pregnancy test will be performed to assess subject eligibility within 72 hours prior to the first administration of IP, if the initial serum pregnancy test did not already occur with 72 hours of dosing (negative results required for IP administration). During the Treatment period urine or serum pregnancy test is allowed.

[3] Hematology assessment includes RBC count, Hb, Hct, MCV, MCH, MCHC, RDW, WBC count with differential, absolute neutrophil count (ANC) and absolute lymphocytes, platelet and reticulocyte absolute values, and circulating erythroblast (nucleated RBC) counts. On dosing days, hemoglobin levels should be measured prior to dosing with IP to ensure Hb levels are within the acceptable ranges to allow luspatercept administration as per Section 7.2 and that luspatercept dose modification rules are followed as outlined in Table 5. Laboratory evaluations may be repeated more frequently if clinically indicated.

[4] Serum chemistry (calcium, magnesium, phosphorus, blood urea nitrogen [BUN], creatinine, creatinine clearance, albumin, total protein, alkaline phosphatase, bilirubin [total, non-conjugated], AST/SGOT, ALT/SGPT, LDH and uric acid). If clinically significant, any or all laboratory evaluations should be reported as AEs and repeated more frequently, if clinically indicated.

[5] For ADA test, additional blood draws are not needed. The test will be performed using the serum collected for PK samples. If ADA test is positive at the time of End of Study, the subject may need to return for additional ADA testing.

[6] DXA scan of the lumbar spine and total hip for bone mineral density (BMD).

[7] Day 22 post-last dose corresponds to the end of Treatment Period.

[8] Calculated doses requiring reconstituted volume greater than 1 mL should be divided equally into two syringes and injected into two separate sites – same anatomical location but opposite side of the body (example left thigh and right thigh). In case of 2 simultaneous injections, the injection sites must be different. In case of dose delays refer to Section 6.2.1 and Section 6.3.1. Only for Dose 1 Day 1, IP administration should be at the end of the Screening/Run-in period, which is at least 12 weeks.

[9] Assessments to be performed if the subject is in this study at Week 96, not applicable if subject does not enter into the LTTP.

[10] During the Long-term Treatment Period, PK and ADA samples will be collected every 6 doses. The maximum ADA monitoring period will be two years from dose 1 day 1 of the double-blind Treatment Period. During the Open-label Phase: for placebo-cross-over subjects, if positive at unblinding, continue every 6 doses up to 2 years from Dose 1 Day 1 of the double-blind Treatment Period; for subjects continuing treatment with luspatercept, only if positive at unblinding, every 6 doses up to 2 years from Dose 1 Day 1 of the double blinded treatment period.. During the Post-Treatment Follow-Up : Before unblinding, ADA monitoring, every 24 weeks (post last dose), at post-treatment follow-up visits will continue for up to two years from Dose 1 Day 1 of the double-blind Treatment Period. After unblinding, ADA monitoring, every 24 weeks (post last dose), at all post-treatment follow-up visits will continue only if subjects' last available ADA is positive and have not reached the 2-year maximum limit for ADA monitoring. . ADA/PK sampling per Investigator's or Sponsor's discretion is allowed and should be recorded as unscheduled visit.

[11] During the Long-term Treatment Period these parameters should be measured by Central Lab. Data to be entered in the eCRF.

[12] MRI for LIC, myocardial iron (T2*) and for spleen volume can be performed on one MRI acquisition if possible.

[13] LTTP will end when all subjects completed 48 weeks of double-blind treatment or discontinue before reaching 48 weeks double-blind treatment, whichever is the earlier date, or at the time the study is unblinded (per DMC recommendation). Only subjects that do not discontinue for reasons as described in Section 11 can enter in the LTTP.

[14] Only for subject who discontinue prior to completion of 48 weeks in the double-blind Treatment Period.

[15] End of Study visit can occur at any time up to at least 156 weeks post last dose.

[16] HCV-RNA test and HBsAg and/or HBVDNA .

[17] +/- 10 day window around for MRI assessments and DXA is allowed (MRI for LIC, myocardiac iron, spleen and LVEF). These assessments to be performed as indicated on specified week, regardless of dose delays. MRIs and DXA scans performed within the 4 weeks prior to the ICF signature can be accepted, if subject does not show any relevant clinical symptoms judged by the Investigator as sufficient reason to repeat these procedures within the Screening/Run-in period and agreed with the Sponsor.

[18] Placebo subject who discontinued the study, but were compliant with the protocol 48 weeks post Dose 1 Day 1, and continued their participation in the Post-treatment Follow-up Period until the time of unblinding, refer to Section 3.1.4.

[19] For all subjects on treatment at the time of Open-label Phase initiation, continue with the appropriate dose number.

[20] For subjects who have documented diagnosis of β-thalassemia in their medical history, by other methodology or do not have a source laboratory report in the medical files, the β-thalassemia genotype can be done at any time during the course of the study.

[21] The same Cardiac Doppler Echocardiography technique should be used within a subject throughout the study duration.

[22] As per standard of care.

[23] Follow-up visits at Weeks 9, 24, 48, 72, 96, 120, 144 and 156 post last dose should be performed by all subjects, regardless if a subject has Early Discontinued or not.

[24] For early discontinuation subjects, these parameters to be reported up to 48 weeks Post Dose 1 Day 1 or Week 9 post last dose whichever is the later date.

[25] In the event the subject receives transfusions at outside institutions during the course of study participation, a subject diary will be available for the subject, in order to capture any transfusions occurring outside the investigational site when there is not an established plan in place.

[26] Subjects are required to see the Investigator at least every 24 weeks to assess for the presence of malignancy and pre-malignancy, as per standard of care.

6. PROCEDURES

Any questions regarding the protocol should be directed to the Celgene Medical Monitor or designee.

All of the protocol required assessments are listed in Section 5, Table 3 with an "X" indicating at which visits the assessments are to be performed. All data obtained from these assessments must be recorded in the subject's source documentation. All visits during the Treatment Period must occur within ± 3 days of the scheduled day. An administrative visit window of ± 5 days is permitted in the Long-term Treatment Period, as well as Open-label Phase. During the Post-treatment Follow-Up Period, visits must occur within ± 7 days of the scheduled day. Procedures are described in detail below.

Subjects must have Hb assessed and results available prior to each study drug administration. If a transfusion occurs prior to dosing, the pre-transfusion Hb should be considered for dosing purposes (ie, Hb ≥ 14 days post- transfusion).

Subjects must have blood pressure assessed prior to each study drug administration. Blood pressure values should be confirmed by means of two readings obtained approximately 10 minutes apart with the subject seated for approximately 10 minutes prior to initial reading. The two readings should be recorded in the source data file.

Safety laboratory analyses and all laboratory assessments will be performed centrally (except as otherwise indicated in Table 3) during the double-blind Treatment Period and Long-term Treatment Period (except otherwise stated in Table 3). Blood or urine samples for local laboratory assessments are allowed in the following circumstances when timely results are needed: eg, randomization, study treatment dosing decisions, assessments between clinic visits, or adverse event. Local laboratory data should be collected in the eCRF if relevant to dose administration, modification and AE, or when no central laboratory results were obtained. During the Open-label Phase all laboratory assessments will be performed locally.

6.1. Screening/Run-in Period

Screening/run-in evaluations will be performed for all subjects to determine study eligibility. These evaluations must be completed within 12 weeks of randomization (refer to Table of Events, Table 3 for further information).

Waivers to the protocol will not be granted during the conduct of this trial, under any circumstances.

Screening laboratory values must demonstrate subject eligibility, but may be repeated within the screening window, if necessary, or subject can be re-screened (refer to Section 6.1.1 for further information on re-screening).

The regularly transfused β-thalassemia subjects must have at least 24 weeks of documented transfusion history available (including Hb levels prior to each transfusion, the number of units transfused, the dates of transfusion) prior to randomization. The 12 weeks of transfusion history will be collected prospectively during the Screening/Run-in Period, and the 12 weeks historical retrospective data will be further recorded in the subject's electronic case report form (eCRF).

The following will be performed at screening/run-in as specified in the Table of Events (Table 3), after informed consent has been obtained:

- Written dated informed consent
- Assessment of inclusion/exclusion criteria for study eligibility
- Demographics (date of birth, sex, race, and ethnicity-if allowed by local regulations)
- Complete medical history: specific information regarding all relevant medical conditions diagnosed (occurring prior to screening) should be recorded including the staging, histology, and any additional details, as needed. Prior ESA history (24 weeks prior to randomization) should also be recorded.
- β-thalassemia genotype (ie, beta and alpha globin mutations, only if not available in subject medical history)
- Hepatitis B & C;
- Iron chelating therapies, including type of iron chelating therapy, period of receive (ie, dates) and dose changes: at least 12 weeks of history before randomization
- Other prior medications/therapies: including those taken ≤ 28 days before screening/run-in, such as surgery, radiation, systemic or any other therapy for the subject's disease
- Prior procedures (including all procedures occurring ≤ 28 days before screening/run-in)
- Transfusion assessment - RBC transfusion history (≥ 24 weeks of history prior to Dose 1 Day 1, ie, 12 weeks of transfusion history will be collected prospectively during the Screening/Run-in Period, and 12 weeks transfusion history will be collected retrospectively), including Hb levels prior to each transfusion, the number of units transfused, the dates of transfusion. If available, the volume transfused in mL at each transfusion and hematocrit (Hct) of the transfused unit should be collected, as well as the age when subject started regular transfusion. If available, additional transfusion history up to 1 year transfusion history can be collected in the eCRF.
    - In the event the subject receives transfusions at outside institutions during the course of study participation, a subject diary will be available for the subject, in order to capture any transfusions occurring outside the investigational site when there is not an established plan in place.
- Adverse event assessment regardless of causality begins when the subject signs the informed consent form, until 9 weeks post last dose. Only, related AE will be collected from 9 weeks until End of Study.
- Malignancy and Pre-Malignancy Reporting:
    - Continuous starting after informed consent signature, regardless of causality reporting occurrence of any case.
    - The occurrence of a new malignancy or pre-malignant lesion will be monitored and collected as an event of interest and should be included as part

of the assessment of adverse events throughout the course of the study. Investigators are to report the development of any new malignancy or pre-malignant lesion as a serious adverse event, regardless of causal relationship to IP (study drug[s] or control), occurring at any time for the duration of the study, from the time of signing the ICF up to and including 156 weeks of follow-up (refer to Section 10.5.2 for additional information).

- Vital signs (including seated blood pressure, temperature, and heart rate). Seated blood pressure should be confirmed by two separate measurements obtained 10 minutes apart - assessment should be performed within 4 weeks prior to Dose 1 Day 1
- Height, weight - assessment should be performed within 4 weeks prior to Dose 1 Day 1
- Eastern Cooperative Oncology Group (ECOG) performance status (Appendix B) - assessment should be performed within 4 weeks prior to Dose 1 Day 1
- 12-lead electrocardiogram (ECG) - assessment should be performed within 4 weeks prior to Dose 1 Day 1 (read locally)
- Cardiac Doppler echocardiography, MUGA or MRI - left ventricular ejection fraction (LVEF)
- Pregnancy testing is required for all female subjects of childbearing potential. Serum beta human chorionic gonadotropin (β-hCG pregnancy test (which must be negative) with a minimum sensitivity of 25 mIU/mL will be performed within 4 weeks prior Dose 1 Day 1.
- For female subjects– menstrual status
- Hematology assessment includes a RBC count, Hb, Hct, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), white blood cell (WBC) count with differential, absolute neutrophil count (ANC), absolute lymphocytes, platelets, - central laboratory assessment should be performed within 4 weeks prior to Dose 1 Day 1. Absolute reticulocytes, and circulating erythroblasts (nucleated RBC) counts should be measured by local laboratory assessment within 4 weeks prior to Dose 1 Day 1
- Serum chemistry includes calcium, magnesium, phosphorus, blood urea nitrogen (BUN), creatinine, creatinine clearance, albumin, total protein, alkaline phosphatase, bilirubin (total, nonconjugated), aspartate aminotransferase (AST)/serum glutamic oxaloacetic transaminase (SGOT), ALT/ serum glutamic pyruvic transaminase (SGPT), lactic dehydrogenase (LDH) and uric acid - central laboratory assessment should be performed within 4 weeks prior to Dose 1 Day 1
- Urinalysis: microalbumin, creatinine, microalbumin/creatinine ratio (morning void; central lab) - central laboratory assessment should be performed within 4 weeks prior to Dose 1 Day 1
- MRI for LIC (T2* or R2, mg/g dw). The same MRI technique should be used within a subject throughout the study duration.

- MRI or abdominal ultrasound for spleen measurement, unless splenectomized. If MRI is used: the spleen volume (cm^3) should be measured in the same acquisition as the MRI for LIC, if possible. If abdominal ultrasound is used for spleen measurement, the longest longitudinal dimension and width should be taken.
- MRI for myocardial iron (T2*; ms)
- Bone mineral density by dual energy x-ray absorptiometry (DXA) scan - lumbar spine and total hip.
- QoL Questionnaire (TranQoL; SF36) - assessment should be performed within 4 weeks prior to Dose 1 Day 1
- HRU
- Serum ferritin – one central laboratory assessment should be performed within 4 weeks prior to Dose 1 Day 1. Record available data from local laboratory assessments on serum ferritin for at least 12 weeks prior to randomization.
- CCI [redacted]

6.1.1. Re-screening

If a subject fails screening, subject can be re-screened as per Investigator discretion. Re-screening more than one time is allowed. A new subject number will be assigned by IRT. All screening assessments performed until the subject is declared to be a screen fail (12 weeks after the ICF signature) can be entered in the eCRF under the new assigned subject ID, if these are within the validity windows indicated as per Table 3, Screening/Run-in, ie:

- All laboratory assessments indicated in the Table 3 "within 4 wks prior Dose 1 Day 1" will be considered as valid if not exceeding 4 weeks of the new planned randomization date. If outside of this window, the assessment should be repeated.
- All other assessments for which there is no restriction indicated in Table 3, will be considered as valid if not exceeding 12 weeks of the new planned randomization date. If outside of this window, the assessment should be repeated.
- However, abnormal assessments that caused ineligibility of the subject must always be repeated.

6.2. Treatment Period

The subject will begin treatment upon acknowledgement of eligibility by the Sponsor. The subject must start treatment approximately 12 weeks from signing the informed consent form (ICF). An administrative window of additional 7 days is allowed for administration of Dose 1. For all subsequent visits, an administrative window of ± 3 days is permitted. If screening assessments are performed within 72 hours of Dose 1 Day 1, safety laboratory assessments do not need to be repeated at Dose 1 Day 1, except hematology and blood chemistry assessments.

Subjects will receive their first SC dose of luspatercept or placebo on Day 1 of each dosing cycle.

Treatment is administered every 21 days, and will occur as described in Section 7.2.

The following evaluations will be performed at the frequency specified in the Table of Events, Table 3. The evaluations should be performed prior to dosing on the visit day, as applicable, unless otherwise specified:

- Iron chelating therapies: record on an ongoing basis (as detailed in Section 6.1).
- Other concomitant medications/therapies: such as surgery, radiation, systemic or any other therapy for the subject's disease - record on an ongoing basis.
- Concomitant procedures - record on an ongoing basis.
- Transfusion assessment - including Hb levels prior to each transfusion, the number of units transfused, the dates of transfusion. If available, the volume transfused in mL at each transfusion and Hct of the transfused unit should also be reported. Record the transfusion assessments on an ongoing basis. In addition, as detailed in Section 6.1, a subject diary will be available (refer to Section 6.1).
- Adverse event assessment - record on an ongoing basis (refer to Section 6.1 and Section 10 for additional information).
- Malignancy and Pre-Malignancy Reporting (refer to Section 6.1 and Section 10.5.2 for additional information)
- Vital signs (as detailed in Section 6.1)
- ECOG performance status
- Weight
- 12-lead electrocardiogram (ECG) – read locally
- Cardiac Doppler echocardiography, MUGA or MRI- left ventricular ejection fraction (LVEF)
- Urine or serum medically supervised pregnancy test with a minimum sensitivity of 25 mIU/mL for FCBP at Day 1 of each dose schedule (with the exception of the first day of dosing in dose schedule 1 if performed during Screening within 72 hours of Day 1)
- For female subjects – menstrual status
- Hematology assessments (as detailed in Section 6.1)
- Serum chemistry (as detailed in Section 6.1)
- Urinalysis: microalbumin, creatinine, microalbumin/creatinine ratio (morning void; central lab)
- Serum erythropoietin
- PK and ADA – refer to Section 6.8 and 6.9
- MRI for LIC (T2* or R2, mg/g dw). The same MRI technique should be used within a subject throughout the study duration.

- MRI or abdominal ultrasound for spleen measurement, unless splenectomized (as detailed in Section 6.1).
- MRI for myocardial iron (T2*; ms)
- Bone mineral density of total hip and lumbar spine by DXA scan(as detailed in Section 6.1)
- QoL self-administered questionnaire TranQoL, SF36
- HRU
- Serum ferritin
- CCI [redacted]
- CCI [redacted]
- Administration/ Accountability of luspatercept/placebo on Day 1 of each dose cycle

6.2.1. Dose Delays in Treatment Period

In case of dose delay (refer to Table 5), the clinical assessments and procedures to be performed include but are not limited to:

- All planned Day 1 assessments should be performed and appropriate samples sent to the central laboratory, regardless of the dose delay.
- If dose delay is due to **an AE**, **a laboratory or vital signs abnormality** (refer to Table 5, Dose Delay, Dose Reduction and Discontinuation Guidelines, Section 7.2.1.2), repeat any clinically indicated assessment by local laboratory at a frequency decided by the Investigator until the subject meets either the treatment administration criteria or treatment discontinuation criteria, as detailed in Section 7.2 and Section 11.1.
- If dose delay is **due to increased hemoglobin level** (refer to Table 5, Dose Delay, Dose Reduction and Discontinuation Guidelines, Section 7.2.1.2), perform hematology on Day 1 of dose delay by central laboratory and then at least weekly by local lab, until the subject meets either the treatment administration criteria or treatment discontinuation criteria, as detailed in Section 7.2 and Section 11.1
- PK/ADA samples should be performed on Day 1 of dose delay and before next dose.
- At the time of IP administration following a dose delay, perform the scheduled dosing visit assessments (laboratory assessments should be performed by central laboratory) as detailed in the Table of Events, Table 3, and Section 6.2, regardless of the duration of the dose delay.

6.3. Long-term Treatment Period

All subjects, whether randomized to luspatercept or placebo, who complete the double-blind treatment period of this study will have the opportunity to enroll in the Long-term Treatment Period and continue to receive IP to which subjects have been initially assigned, as described in Section 3.1.3. An administrative visit window of ± 5 days is permitted.

During the Long-term Treatment Period all laboratory assessments should be measured by central laboratory. Data are to be entered in the eCRF.

The assessments and procedures that will be performed during the Long-term Treatment Period are outlined in the Table of Events (see Table 3) and include but are not limited to:

- Iron chelating therapies: record on an ongoing basis (as detailed in Section 6.1).
- Other concomitant medications/therapies: such as surgery, radiation, systemic or any other therapy for the subject's disease - record on an ongoing basis.
- Concomitant procedures - record on an ongoing basis.
- Transfusion assessment - record on an ongoing basis (as detailed in Section 6.1). In addition, as detailed in Section 6.1, a subject diary will be available (refer to Section 6.1)
- Adverse event assessment - record on an ongoing basis (refer to Section 6.1 and Section 10 for additional information).
- Malignancy and Pre-Malignancy Reporting (refer to Section 6.1 and Section 10.5.2 for additional information)
- Vital signs (as detailed in Section 6.1)
- Weight
- Cardiac Doppler echocardiography, MUGA or MRI - left ventricular ejection fraction (LVEF)
- Urine or serum medically supervised pregnancy test with a minimum sensitivity of 25 mIU/mL for FCBP at Day 1 of each dose schedule.
- For female subjects – menstrual status
- Hematology (as detailed in Section 6.1)
- Serum chemistry (as detailed in Section 6.1)
- Urinalysis: microalbumin, creatinine, microalbumin/creatinine ratio (morning void; central lab)
- PK and ADA (if applicable) – refer to Sections 6.8 and 6.9
- Serum ferritin
- MRI for LIC (T2* or R2, mg/g dw). The same MRI technique should be used within a subject throughout the study duration.
- MRI or abdominal ultrasound for spleen measurement, unless splenectomized (as detailed in Section 6.1).
- MRI for myocardial iron (T2*; ms)
- Bone mineral density of total hip and lumbar spine by DXA scan(as detailed in Section 6.1)

- QoL self-administered questionnaire TranQol; SF36
- HRU
- Administration / Accountability of luspatercept on Day 1 of each dose

6.3.1. Dose Delays in Long-term Treatment Period

In case of dose delay (refer to Table 5), the clinical assessments and procedures to be performed include but are not limited to:

- All planned Day 1 assessments should be performed regardless of the dose delay.
- If dose delay is due to **an AE, a laboratory or vital signs abnormality** (refer to Table 5, Dose Delay, Dose Reduction and Discontinuation Guidelines, Section 7.2.1.2), repeat any clinically indicated assessment by local laboratory at a frequency decided by the Investigator until the subject meets either the treatment administration criteria or treatment discontinuation criteria, as detailed in Section 7.2 and Section 11.1.
- If dose delay is **due to increased hemoglobin level** (refer to Table 5, Dose Delay, Dose Reduction and Discontinuation Guidelines, Section 7.2.1.2), perform hematology on Day 1 of dose delay by central laboratory and then at least weekly by local lab until the subject meets either the treatment administration criteria or treatment discontinuation criteria, as detailed in Section 7.2 and Section 11.1.
- At the time of IP administration following a dose delay, perform the scheduled dosing visit assessments (laboratory assessments should be performed by central laboratory) as detailed in the Table of Events, Table 3, and Section 6.3, regardless of the duration of the dose delay.

6.4. Open-label Phase

During the Open-label Phase, the following evaluations will be performed at the frequency specified in the Table of Events, Table 3. The evaluations should be performed prior to dosing on the visit day, as applicable, unless otherwise specified. An administrative visit window of ± 5 days is permitted.

Only subjects who, at the time of unblinding, are in Post-treatment Follow-Up and are on placebo, will perform the following assessments and procedures **in addition to the procedures and assessments that will be performed by subjects who are continuing the treatment with luspatercept after the unblinding** of the study:

- Assessment of eligibility (only subjects who are in the Post-treatment Follow-up who were treated with placebo during the double-blind treatment and discontinued the IP). Inclusion criteria numbers 6 and 7 and exclusion criteria numbers 1-10, 15-17 and 21 must be met at Dose 1 Day 1
- Iron chelating therapies: including type of iron chelating therapy, period of treatment (ie, dates) and dose changes 24 weeks prior to Dose 1 Day 1 with luspatercept in the Open label Phase and record on ongoing basis (as detailed in Section 6.1).

- Prior medications/therapies: 24 weeks prior to Dose 1 Day 1 of luspatercept
- Prior procedures 24 weeks prior to Dose 1 Day 1 of luspatercept
- Transfusion assessment – 24 weeks RBC transfusion history prior to Dose 1 Day 1 of the Open-label Phase of luspatercept including Hb levels prior to each transfusion, the number of units and or volumes transfused, and the dates of transfusion. If available, the Hct of the transfused unit should be collected, as well as the age when the subject started regular transfusion. These retrospective data will be recorded in the subject's electronic case report form (eCRF).
- Serum ferritin: 24 weeks prior to Dose 1 Day 1 of luspatercept.
- ECOG performance status (Appendix B)
- 12-Lead Electrocardiogram – read locally
- Females: Serum medically supervised pregnancy test with a minimum sensitivity of 25 mIU/mL for FCBP at Day 1 Dose 1 of luspatercept
- Serum chemistry (as detailed in Section 6.1)
- Urinalysis: microalbumin, creatinine, microalbumin/creatinine (as detailed in Section 6.1)

All subjects, ie, subjects who are continuing the treatment with luspatercept after the unblinding of the study, and subjects who are starting the treatment with luspatercept after received placebo in the double-blind treatment, will perform the following assessments and procedures:

- Iron chelating therapies: record on an ongoing basis (as detailed in Section 6.1).
- Concomitant medications/therapies: such as surgery, radiation, systemic or any other therapy for the subject's disease - record on ongoing basis.
- Concomitant procedures - record on ongoing basis.
- Transfusion assessment - record on an ongoing basis (as detailed in Section 6.1). In addition, as detailed in Section 6.1, a subject diary will be available (refer to Section 6.1)
- Adverse event assessment - record on an ongoing basis as detailed in Section 6.1 and Section 10.
- Malignancy and Pre-Malignancy Reporting (refer to Section 6.1 and Section 10.5.2 for additional information)
- Vital signs (as detailed in Section 6.1)
- Weight
- Female subjects of childbearing potential: Urine or serum medically supervised pregnancy test with a minimum sensitivity of 25 mIU/mL for FCBP at Day 1 of each dose
- Female subjects of childbearing potential: menstrual status
- Hematology (as detailed in Section 6.1)
- ADA (if applicable) – refer to Section 6.9 and Table 3

- HRU
- Administration of luspatercept on Day 1 of each dose
- Only at Week 96 post first dose, if applicable, ie, for subjects who entered Open-label Phase prior to reaching Week 96 in the Long-term Treatment Period:
    - MRI for LIC (mg/g dw) (as detailed in Section 6.1) - only at week 96 post first dose, if applicable.
    - MRI for myocardial iron (T2*; ms) - only at week 96 post first dose, if applicable.
    - MRI or abdominal ultrasound for spleen measurement (as detailed in Section 6.1) - only at week 96 post first dose, if applicable.
    - Bone imaging DXA scan (as detailed in Section 6.1) - only at week 96 post first dose, if applicable.

6.4.1. Dose Delays in Open-label Treatment Period

In case of dose delay (refer to Table 5), the clinical assessments and procedures to be performed include but are not limited to:

- If dose delay is **due to increased hemoglobin level** (refer to Table 5, Dose Delay, Dose Reduction and Discontinuation Guidelines, Section 7.2.1.2), perform hematology on Day 1 of dose delay by central laboratory and then at least weekly by local laboratory until the subject meets either the treatment administration criteria or treatment discontinuation criteria, as detailed in Sections 7.2.1.2, 7.2.1.3 and 11.1
- If dose delay is **due to an AE, laboratory or vital signs abnormality** (refer to Table 5, Dose Delay, Dose Reduction and Discontinuation Guidelines, Section 7.2.1.2), repeat any clinically indicated assessment by local laboratory at a frequency decided by the Investigator until the subject meets either the treatment administration criteria or treatment discontinuation criteria, as detailed in Sections 7.2.1.3 and 11.1

6.5. Treatment Discontinuation

Reasons for treatment discontinuation are provided in Section 11. An end of treatment evaluation will be performed for subjects who are withdrawn from treatment for any reason as soon as possible after the decision to permanently discontinue treatment has been made.

Subjects who discontinue early from treatment will proceed to the Treatment Discontinuation visit within 22 days of the last dose of study drug, or, soon after the decision to discontinue the study drug, if this occurred more than 22 days after the last dose of study drug.

The assessments and procedures that will be performed at Treatment Discontinuation are outlined in the Table of Events (see Table 3) and include but are not limited to:

- Iron chelating therapies: record on an ongoing basis (as detailed in Section 6.1).
- Other concomitant medications/therapies: such as surgery, radiation, systemic or any other therapy for the subject's disease - record on an ongoing basis.

- Concomitant procedures - record on an ongoing basis.
- Transfusion assessment - record on an ongoing basis (as detailed in Section 6.1). In addition, as detailed in Section 6.1, a subject diary will be available (refer to Section 6.1)
- Adverse event assessment - record on an ongoing basis (refer to Section 6.1 and Section 10 for additional information).
- Malignancy and Pre-Malignancy Reporting (refer to Section 6.1 and Section 10.5.2 for additional information)
- Vital signs (as detailed in Section 6.1)
- Weight
- Urine or serum medically supervised pregnancy test with a minimum sensitivity of 25 mIU/mL for FCBP
- For female subjects only - menstrual status
- Hematology (as detailed in Section 6.1)
- Serum chemistry (as detailed in Section 6.1)
- Urinalysis: microalbumin, creatinine, microalbumin/creatinine ratio (morning void; central lab)
- 12-lead electrocardiogram (ECG) – read locally
- PK – refer to Section 6.8
- ADA – refer to Section 6.9
- Serum erythropoietin
- MRI for LIC (T2* or R2, mg/g dw). The same MRI technique should be used within a subject throughout the study duration.
- MRI or abdominal ultrasound for spleen measurement, unless splenectomized (as detailed in Section 6.1)
- MRI for myocardial iron (T2*; ms)
- Bone mineral density of total hip and lumbar spine by DXA scan (as detailed in Section 6.1)
- Serum ferritin
- CCI [redacted]
- CCI [redacted]
- HRU

6.6. Post-treatment Follow-up Period

At the end of the Treatment Period, Long-term Treatment Period, or Open-label Phase, as applicable, all subjects who discontinue treatment will be followed for an additional 156 weeks from last dose of IP received. Subjects will be monitored on week 9, followed by 24, 48, 72, 96, 120, 144 after the last dose up to week 156. End of study visit, will coincide with week 96, refer to Section 6.7. A visit window of ± 7 days is permitted.

The assessments and procedures that will be performed during this period are outlined in the Table of Events (see Table 3) and include but are not limited to:

Only for Follow-Up Week 9 post last dose, the below assessments will be performed:

- Iron chelating therapies: record on an ongoing basis up to week 9 Post-last dose (as detailed in Section 6.1).
- Other concomitant medications/therapies: such as surgery, radiation, systemic or any other therapy for the subject's disease - record on an ongoing basis up to week 9 Post-last dose..
- Concomitant procedures - record on an ongoing basis up to week 9 Post-last dose..
- Transfusion assessment - record on an ongoing basis up to week 9 Post-last dose (as detailed in Section 6.1). In addition, as detailed in Section 6.1, a subject diary will be available (refer to Section 6.1).
- Adverse event assessment regardless of causality- record on an ongoing basis up to Week 9 Post-last dose, and only related AE from Week 9 Post-last dose up to End of Study (refer to Section 6.1 for additional information).
- Malignancy and Pre-Malignancy Reporting - regardless of causality until End of Study. (refer to Section 6.1 and Section 10.5.2 for additional information)
- Vital signs (as detailed in Section 6.1)
- Weight
- Urine or serum medically supervised pregnancy test with a minimum sensitivity of 25 mIU/mL for FCBP
- For female subjects only - menstrual status
- Hematology (as detailed in Section 6.1)
- Serum chemistry (as detailed in Section 6.1)
- Urinalysis: microalbumin, creatinine, microalbumin/creatinine ratio (morning void; central lab)
- PK and ADA – refer to Sections 6.8 and 6.9
- Serum ferritin
- HRU

Post-treatment Follow-up monitoring at Week 24, 48, 72, 96, 120, 144 post last dose – assessments and procedures, that will be performed during this period are outlined in the Table of Events (see Table 3) and include but are not limited to:

- Adverse event assessment - record all AEs regardless of causality on an ongoing basis up to week 9 Post-last dose, and only related AE from week 9 Post-last dose up to End of Study (refer to Section 6.1 for additional information).
- Malignancy and Pre-Malignancy Reporting - regardless of causality as SAEs until End of Study. Subjects are required to see the Investigator at least every 24 weeks to assess for malignancy and pre-malignancy, as per standard of care (refer to Section 10.5.2 for additional information)

6.6.1. Early Discontinuation Follow-up Period

Subjects who discontinue before completing the double-blind treatment period (48 weeks) will continue to be monitored on week 9, followed by week , 24, 48, 72, 120, 144 after the last dose.

The assessments and procedures that will be performed during this Early Discontinuation Follow-up Period are outlined in the Table of Events (see Table 3) and include but are not limited to:

- Iron chelating therapies: record on an ongoing basis (as detailed in Section 6.1) - this parameters is to be reported up to 48 weeks Post Dose 1 Day 1 or Week 9 post last dose whichever is the later date.
- Other post-treatment medications/therapies: such as surgery, radiation, systemic or any other therapy for the subject's disease - record on an ongoing basis - these parameters are to be reported up to 48 weeks Post Dose 1 Day 1 or Week 9 post last dose whichever is the later date.
- Post-treatment procedures - record on an ongoing basis - these parameters are to be reported up to 48 weeks Post Dose 1 Day 1 or Week 9 post last dose whichever is the later date.
- Transfusion assessment - record on an ongoing basis (as detailed in Section 6.1). In addition, as detailed in Section 6.1, a subject diary will be available (refer to Section 6.1). - these parameters are to be reported up to 48 weeks Post Dose 1 Day 1 or Week 9 post last dose whichever is the later date.
- Adverse event assessment - record AEs regardless of causality on an ongoing basis until 9 weeks post last dose, related AE to be reported from week 9 Post-last dose until End of Study.
- Malignancy and Pre-Malignancy Reporting - regardless of causality as SAEs until End of Study. Subjects are required to see the Investigator at week 9 and at least every 24 weeks thereafter to assess for malignancy and pre-malignancy, as per standard of care (refer to Section 10.5.2 for additional information)
- MRI for LIC (T2* or R2, mg/g dw). The same MRI technique should be used within a subject throughout the study duration.

- MRI for abdominal ultrasound spleen measurement, unless splenectomized (as detailed in Section 6.1)
- MRI for myocardial iron (T2*; ms).
- Bone mineral density of total hip and lumbar spine by DXA scan (as detailed in Section 6.1).
- HRU - this parameter is to be reported up to 48 weeks Post Dose 1 Day 1 or Week 9 post last dose whichever is the later date.
- Vital signs (as detailed in Section 6.1)
- Weight

6.7. End of Study Visit

Reasons for premature End of Study are provided in Section 11.2. The End of Study visit is the final scheduled visit for this study and can coincide with week 156 Post last dose.

The assessments and procedures that will be performed during the End of Study are outlined in the Table of Events (see Table 3) and include but are not limited to:

- Adverse event assessment - record AEs regardless of causality on an ongoing basis up to week 9 Post-last dose, and only related AE up to End of Study (refer to Section 6.1 for additional information).
- Malignancy and Pre-Malignancy Reporting - regardless of causality as SAE until End of Study (refer to Section 10.5.2 for additional information).

The reason for discontinuation should be recorded in the eCRF and in the source documents.

6.8. Pharmacokinetics

Serum samples will be collected to analyze luspatercept concentrations (PK) in all subjects as indicated in Table 3. Collection, processing, and shipping procedures for blood PK samples are provided in the study reference guide.

- Treatment Period: Doses 1 to 16: at predose (Dose 1 Day 1, must be collected before the first dose), Dose 2 Day 1, Dose 3 Day 1, Dose 4 Day 1, Dose 5 Day 1, Dose 6 Day 1, Dose 6 Day 8, Dose 6 Day 15, Dose 8 Day 1, Dose 10 Day 1, Dose 12 Day 1, Dose 14 Day 1, and Dose 16 Day 1
- Long-term Treatment Period: every 6 doses. ADA/PK sampling per Investigator's or Sponsor's discretion is allowed and should be recorded as an unscheduled visit

6.9. Anti-Drug Antibody

Serum samples will be collected for assessment of anti-drug antibodies (ADA) in all subjects. The maximum ADA monitoring period will be two years from dose 1 day 1 of double-blind Treatment Period. An additional blood draw is not needed for the anti-luspatercept antibody test.

The anti-luspatercept antibody test will be conducted for the following visits utilizing the PK samples obtained at the same visit:

- Treatment Period: Doses 1 to 16: Predose (Dose 1 Day 1, must be collected before the first dose), Dose 2 Day 1, Dose 4 Day 1, Dose 6 Day 1, Dose 8 Day 1, Dose 12 Day 1, Dose 16 Day 1.
- Long-term Treatment Period: every 6 doses.
- Open Label Phase: for placebo-cross-over subjects, only if positive at unblinding, continue every 6 doses up to 2 years from Dose 1 Day 1 of the double-blind Treatment Period. For subjects continuing treatment with luspatercept, only if positive at unblinding, every 6 doses up to 2 years from Dose 1 Day 1 of the double blinded treatment period..
- Post-treatment Follow-up (starting at Follow-up Week 9):
  - Before unblinding: every 24 weeks post last dose until the unblinding for up to two years from Dose 1 Day 1 of double-blind Treatment Period.
  - After unblinding, ADA monitoring every 24 weeks post last dose, only if subjects' last available ADA is positive and they have not reached the 2-year maximum limit for ADA monitoring.

ADA/PK sampling per Investigator's or Sponsor's discretion is allowed and should be recorded as an unscheduled visit.

CCI [redacted]

6.11. Quality of Life

- TranQoL – Subjects will be asked to complete the quality of life questionnaire TranQoL at screening, and at Weeks 12, 24, 36 and 48 post Day 1 Dose 1, and every 12 weeks during the Long-term Treatment Period.
- SF-36 - Subjects will be asked to complete the quality of life questionnaire SF-36 at screening, and at Weeks 12, 24, 36 and 48 post Day 1 Dose 1, and every 12 weeks during the Long-term Treatment Period.

6.12. Screen Failures

For all subjects determined to be screen failures, the following information is to be captured in the subject's source documents and appropriate eCRF page(s): the date informed consent

document (ICD) was signed, demographics, the reason subject did not qualify for the study, and the Investigator's signature for the eCRF pages. The adverse events experienced by screen failure subjects will be collected from the date of signing consent to the day the subject is confirmed as screen failure. Relevant information will also be recorded on the Screening Log.

7. DESCRIPTION OF STUDY TREATMENTS

7.1. Description of Investigational Product(s)

Luspatercept will be provided by the Sponsor. Luspatercept for injection is formulated as a sterile, preservative-free, lyophilized cake/powder. Luspatercept for injection is available in 2 strengths, and when reconstituted, consists of 50 mg/mL luspatercept in a 10 mM citrate buffer-based solution (10 mM citrate, pH 6.5, 9% sucrose, 0.02% polysorbate 80). The drug product is packaged in a 3 mL glass vial in the following strengths:

- 25 mg/vial: The 25 mg/vial presentation contains 37.5 mg of Luspatercept protein. After reconstitution with 0.68 mL water for injection (WFI), each single-use vial will deliver at least 0.5 mL of 50 mg/mL luspatercept (25 mg)
- 75 mg/vial: The 75 mg/vial presentation contains 87.5 mg of Luspatercept protein. After reconstitution with 1.6 mL WFI, each single-use vial will deliver at least 1.5 mL of 50 mg/mL luspatercept (75 mg)

The recommended storage condition for luspatercept for injection (25 mg/vial and 75 mg/vial; lyophilized powder formulation) is 2-8°C. It is recommended that the reconstituted luspatercept for injection, at room temperature, be administered immediately. However, it may be held for up to 10 hours at 2°C to 8°C.

If not used immediately, the total in-use time of the reconstituted luspatercept for injection, from reconstitution to administration, must not exceed 10 hours.

Samples of luspatercept drug product, held at the recommended storage condition, have been shown to be stable through the labeled shelf-life.

Placebo to be used in the study will be sterile normal saline (0.9% sodium chloride for injection) administered as a SC injection. Sterile, normal saline will be prepared in syringes by the investigational site's designated individuals to match the active syringe. The doctor and subject will be blinded. The manufacturer's directions for placebo storage and handling are to be followed, as are standard clinical practices for ensuring sterility of the placebo.

7.2. Treatment Administration and Schedule

Luspatercept or placebo will be administered as a subcutaneous (SC) injection to subjects by the study staff at the clinical site and administration will be documented in the subject's source record. Subcutaneous injections will be given in the upper arm, thigh, and/or abdomen. Calculated doses requiring reconstituted volume greater than 1.2 mL should be divided into separate similar volume injections across two separate sites using the same anatomical location but on opposite sides of the body (example left thigh and right thigh). The maximum volume per SC injection should not exceed 1.2 mL. The maximum total dose per administration should not exceed 120 mg, which results in 2.4 mL maximum total volume after reconstitution. The injection sites can be rotated according to Investigator judgment, and the injections can be given in the following order as needed, for example: 1) right upper arm, 2) left upper arm, 3) right upper thigh, 4) left upper thigh.

Subjects will be assigned to treatment as per one of the following regimens:

- Luspatercept starting dose level 1 mg/kg SC once every 21 days
- Placebo SC once every 21 days

The investigational product should be administered according to the following criteria:

- **Pre-treatment/ pre-transfusion** Hb value is < 11.5 g/dL and increase of Hb ≤ 2.0 g/dL compared to the pre-dose Hb of Day 1 of the previous treatment dose cycle, and
- Any related adverse events must be < Grade 2 according to NCI CTCAE criteria (Appendix C)
- WBC < 3 x baseline. Corrected WBC values should be used to establish the baseline WBC. Baseline is equal to the highest WBC value between Screening WBC and Study Dose 1 Day 1.
- Leukopenia, neutropenia and/or thrombocytopenia should be < Grade 3, as per NCI CTCAE criteria (Appendix C)
- < 50 % increase from baseline in transfusion burden in combination with shift from baseline (worsening) of < 2 grades in leukopenia, neutropenia or thrombocytopenia

Please refer to Table 5 for guidelines on dose modifications and dose delays.

Subjects must have Hb assessed and results available prior to each study drug administration. If a transfusion occurred prior to dosing, the pre-transfusion Hb should be considered for dosing purposes (ie, Hb ≥ 14 days post- transfusion).

Subjects must have blood pressure assessed prior to each study drug administration. Blood pressure values should be confirmed by means of two readings obtained approximately 10 minutes apart with the subject seated for approximately 10 minutes prior to initial reading.

Safety laboratory analyses and all assessments will be performed centrally (except otherwise stated in Table 3) during the double-blind Treatment Period. However, in addition to the blood draw or urine sampling for central analysis, blood draw or urine sampling for local laboratory assessments are only allowed in the following circumstances: cases when timely results are needed - eg, randomization, study treatment dosing decisions, hematology assessments between clinic visits, adverse event. Local laboratory data should be collected in the eCRF if relevant to dose administration, modification and AE, or when no central lab results were obtained.

Please refer to Table 5 for guidelines on dose modifications and dose delays.

7.2.1. Dose Modifications: Dose Titration, Dose Reduction and Dose Delay

7.2.1.1. Dose Titration

Subjects may be dose-titrated to 1.25 mg/kg, but the maximum total dose should not exceed 120 mg, during the treatment period as well as during the Long-term Treatment Period and Open-label Phase, as long as a dose delay or reduction is not required (see Section 7.2.1.2). The dose titration criteria are defined as follows:

- Transfusion reduction over at least two dose cycles (~6 weeks) is < 33%, compared to the transfusion burden (units/wk) at baseline, or
- Transfusion reduction over at least two dose cycles (~6 weeks) is ≥ 33 but ≤50% compared to baseline, at the discretion of the Investigator.

After safety and efficacy data review, the sponsor may allow dose titration following special requests, such as, but not limited to, subjects who have been dose reduced once, but lost the response to treatment.

Starting dose with dose titration and reductions are presented below for reference (Table 4).

Table 4: Starting Dose Level with Dose Reductions and Dose Titration

3rd Dose Reduction (~ 25 %)	2nd Dose Reduction (~ 25 %)	1st Dose Reduction (~ 25 %)	Starting Dose Level	1st Dose Titration
0.45 mg/kg	0.6 mg/kg	0.8 mg/kg	1.0 mg/kg	1.25 mg/kg

7.2.1.2. Dose Delay and Dose Reduction

Dose delay of luspatercept from the planned dosing schedule may occur due to increased hemoglobin or adverse events. Table 5 provides guidelines for dose modifications and dose delay.

Subjects can experience a dose reduction based on the change in mean Hb level (Hb not influenced by a transfusion) with respect to the last dose, as well as related adverse events, as detailed in Table 5. Hemoglobin not influenced by a transfusion should be considered for dosing, delays and discontinuation actions related to luspatercept. Hemoglobin not influenced by a transfusion is considered a Hb within 14 days post-transfusion. If a subject is experiencing a dose delay due to Hb increase, Hb measurement should occur every week.

If dose delay is 15 weeks or longer from the previous dose administered, including cases of elective surgery/hospitalization, the treatment should be discontinued. Dose delay for AE unrelated to the study drug, as per discretion of the Investigator are allowed. Assessments to be performed during the dose delay period are detailed in Section 6.

Celgene or its authorized representative should be notified of dose modification or interruption within 24 hours.

Dose reduction and dose delays guidelines are detailed in Table 5.

Table 5: Dose Delay, Dose Reduction and Discontinuation Guidelines:

Event at the Day of Dosing	Action
Any related AE = Grade 2[a]	Dose delay[c] until resolved to ≤ Grade 1 or baseline
Any related AE ≥ Grade 3[a]	Dose delay[c] until resolved to ≤ Grade 1 or baseline, and then reduce dose by 25%[d]

Table 5: Dose Delay, Dose Reduction and Discontinuation Guidelines: (Continued)

Event at the Day of Dosing	Action
> 2 dose reductions due to related AE[a]	Discontinue treatment
Increase in Hb >2.0 g/dL compared to pre-dose Hb of Day 1 of the previous treatment dose cycle[b]	Reduce dose by 25%[d] if ΔHb not influenced by RBC transfusions
Hb ≥ 11.5 g/dL[b]	Dose delay until Hb ≤ 11.0 g/dL
Worsening of Anemia	
A ≥ 50 % increase from baseline in transfusion burden in combination with an unexplained shift from baseline (worsening) of ≥ 2 grades[i] in leukopenia, neutropenia or thrombocytopenia[h].	Dose delay[c] and repeat WBC, neutrophils and platelets weekly for two consecutive weeks. • If WBC, neutrophils and platelets are resolved to ≤ Grade 1 or baseline, continue with dosing at the same dose level. • If WBC, neutrophils and platelets are Grade 2 and above baseline, then continue with dose delay until resolution to ≤ Grade 1 and evaluate for alternative explanations of cytopenia as per standard clinical practice. • If shift (worsening) of ≥ 2 grades is maintained for ≥ 14 days or thrombocytopenia does not improve to < Grade 2 within 14 days and no alternative explanation is identified, then perform bone marrow assessment. o If hematologic malignancy is confirmed, discontinue treatment[g] o If hematologic malignancy is not confirmed, then discuss future dosing with medical monitor.
WBC	
WBC count[e] ≥2X baseline in the absence of an associated condition (eg, infection or concomitant corticosteroid use)	**Continue treatment, repeat WBC within 1 week** • **If repeat WBC remains ≥ 2 X above baseline Investigator should assess the cause of increase to exclude hematologic malignancy as per standard clinical practice.** • **If hematologic malignancy is confirmed, discontinue treatment.[g]**
WBC count[e] ≥ 3X baseline	**Dose delay[c] with weekly WBC monitoring until WBC count <3 X baseline.** • **Investigator should assess the cause of increase per standard clinical practice to exclude hematologic malignancy.** • **If hematologic malignancy is confirmed, discontinue treatment.[g]**

Table 5: Dose Delay, Dose Reduction and Discontinuation Guidelines: (Continued)

Event at the Day of Dosing	Action
Grade ≥ 3 Leukopenia, neutropenia and/or thrombocytopenia[i]	**Dose delay[c] and, repeat assessment weekly for two consecutive weeks** • **Investigator should assess the cause of cytopenia per standard clinical practice to exclude hematologic malignancy.** • **If WBC, neutrophils and platelets resolved to ≤ Grade 1, continue with dosing at the same dose level.** • **If WBC, neutrophils and platelets ≥ Grade 2 and above baseline sustained for ≥ 14 days perform bone marrow assessment.** o **If hematologic malignancy is confirmed, discontinue treatment[g]** o **If hematological malignancy is not confirmed, then discuss future dosing with a medical monitor.**
Grade 3 Leukocytosis[f]	**Discontinue treatment**

[a] Possibly, probably or definitely related.
[b] Based on the **pre-treatment** Hb value not influenced by transfusion (ie, ≥ 14 days post- transfusion); Hb should be rechecked weekly during dose delay.
[c] If dose delay is 15 weeks or longer from the prior dose administration, treatment should be discontinued.
[d] Refer to Table 4 for dose reductions.
[e] Central laboratory corrected WBC values must be used for confirming the Investigator decision, which may be based on the local lab result. The baseline WBC is defined as highest WBC value between Screening WBC and Study Dose 1 Day 1.
[f] Grade 3 Leukocytosis (as per CTCAE, ie, WBC above 100K) should be based on Central Laboratory values, as applicable.
[g] For full list of events triggering discontinuation, see Section 11 of the protocol.
[h] For reference, as per CTCAE : Grade 1 Leukopenia : < LLN – 3000/mm3; Grade 1 Neutropenia : < LLN – 1500/mm3; Grade 1 Thrombocytopenia :< LLN – 75'000/mm3; Grade 2 Leukopenia : < 3000 – 2000 / mm3; Grade 2 Neutropenia : < 1500 – 1000 / mm3; Grade 2 Thrombocytopenia :< 75'000 – 50'000 / mm3.
[i] Grade 3 Leukopenia (as per CTCAE, < 2000 - 1000/mm3), Neutropenia (as per CTCAE, ie, < 1000 - 500/mm3) and Thrombocytopenia (< 50,000 - 25,000/mm3).

7.2.1.3. Overdose

Overdose, as defined for this protocol, refers to luspatercept dosing only. On a per dose basis, an overdose is defined as the following amount over the protocol-specified dose of luspatercept assigned to a given subject, regardless of any associated adverse events or sequelae.

SC 10% over the protocol-specified dose

On a schedule or frequency basis, an overdose is defined as anything more frequent than the protocol required schedule or frequency. Complete data about drug administration, including any overdose, regardless of whether the overdose was accidental or intentional, should be reported in the eCRF. See Section 10.1 for the reporting of adverse events associated with overdose.

7.3. Method of Treatment Assignment

The treatment assignment (randomization) will occur at the end of the Screening Period, once all the required screening procedures have been completed and all required data have been submitted to the Sponsor or its authorized representative. Upon receiving acknowledgment of subjects' eligibility from the Sponsor or its authorized representative, subject can be assigned for treatment using IRT. This study will utilize the IRT for enrollment.

Designated research personnel at each investigational site will be assigned password protected, coded identification numbers which gives them the authorization to call into IRT to enroll subjects. For drug assignment at each dose start and in the event of any dose reduction, dose titration site staff must contact IRT to record the new dose level and obtain the new study treatment assignment.

The relationship of the randomization number to the subject ID number will be described by a randomization algorithm. The randomization algorithm will be employed by the IRT system to assign a subject to a treatment based on the prespecified rules, such us double blind study, stratified randomization with randomization ratio active vs placebo on a 2:1; subjects will be placed into the appropriate stratum per the responses/data entered/collected for questions collecting stratification and based on the combination of these data points, the IRT will place the subject in the next available slot within the appropriate stratum for that subject. The IRT will be utilized to ensure an equal weight central randomization based on randomization method according to stratification factor defined in Section 3.1, Study Design. The randomization number corresponds to a particular treatment arm within a stratum. The randomization number, by itself, will not unblind a user to the subject's treatment. The randomization number should be coupled with all the unblinded code information, in order the subject to become unblinded.

7.4. Packaging and Labeling

The label(s) for luspatercept will include Sponsor name, address and telephone number, the protocol number, IP name, dosage form and strength (where applicable), amount of IP per container, lot number, expiry date (where applicable), medication identification/kit number, dosing instructions, storage conditions, and required caution statements and/or regulatory statements as applicable. Additional information may be included on the label as applicable per local regulations.

7.4.1. Blinding

For this trial, all subjects, study site staff and Celgene Corporation representatives with the exception of designated individuals (eg, the pharmacist at the investigational site), will remain blinded to all treatment assignments until all subjects completed 48 weeks of double-blind treatment or discontinue before reaching 48 weeks double-blind treatment, whichever is the earlier date, or at the time the study is unblinded (per DMC recommendation) and the data base is locked.

The designated site individual (for example the pharmacist) at the investigational site will use a syringe (that exactly matches the syringe used for reconstituted luspatercept) and sterile normal saline (0.9% sodium chloride for injection) to prepare a matching placebo. Thus, the designated

site individual at the Investigational site will be unblinded and will give Investigators and their staff luspatercept and placebo in a blinded manner.

Randomization, drug dispensing, dose reduction/titration, and drug discontinuation will be accomplished by an IRT system. Authorized site personnel must contact the IRT for randomization, study drug assignment at the beginning of each cycle, to register dose reductions or titrations, and treatment discontinuation. Confirmation of each call will be sent to the investigational site and Celgene.

For emergency unblinding refer to Section 12.2.

7.5. Investigational Product Accountability and Disposal

Accountability for study drug that is administered during the course of the study is the responsibility of the Investigator or designee. Investigational clinical supplies must be received by a designated person at the clinical site and kept in a secure and temperature-controlled location. The investigational site must maintain accurate records demonstrating dates and amounts of study drug received, to whom it was administered (subject-by-subject accounting), and accounts of any luspatercept accidentally or deliberately destroyed or returned. Accurate recording of all IP administration will be made in the appropriate section of the subject's eCRF and source documents. Unless otherwise notified, all vials of study drug, both used and unused, must be saved for drug accountability. The used vials may be discarded, per the institution's standard practice, after drug accountability has been completed by the monitor. The Investigator must return all unused vials of study drug to the Sponsor at the end of the study, or the study drug may be destroyed at the clinical site with the permission of the Sponsor. For either scenario, the outcome must be documented on the drug accountability log. The Sponsor will provide direction for the outcome of all unused vials.

Celgene (or designee) will review with the Investigator and relevant site personnel the process for investigational product return, disposal, and/or destruction including responsibilities for the site versus Celgene (or designee).

7.6. Investigational Product Compliance

Study drug will be administered as a SC injection at the clinical site by the study staff. Monitoring for subject compliance with the treatment regimen is therefore unnecessary.

Accurate recording of all IP administration will be made in the appropriate section of the subject's eCRF and source documents.

The Investigator or designee is responsible for accounting for all IP that is administered during the course of the study.

8. CONCOMITANT MEDICATIONS AND PROCEDURES

During screening and throughout the study subjects may take stable doses of medications for chronic conditions that are not specifically excluded by the protocol (see Sections 4.2 Inclusion Criteria and 4.3 Exclusion Criteria). The Investigator should consult the medical monitor regarding any questions about whether a new medication or dosage of existing medication would require the patient to discontinue from the study.

Prior/Concomitant medications will be collected beginning at study Screening/Run-in Period and will include all medications taken within 12 weeks prior to Dose 1 Day 1.

For information regarding other drugs that may interact with IP and affect its metabolism, pharmacokinetics, or excretion, please see the Investigators Brochure and/or local package insert.

8.1. Permitted Concomitant Medications and Procedures

8.1.1. General Concomitant Medication Usage

During screening and throughout the study subjects may take stable doses of medications for chronic conditions that are not specifically excluded by the protocol (see Section 4.3 Exclusion Criteria).

The Investigator should consult the medical monitor regarding any questions about whether a new medication or dosage of existing medication would require the patient to discontinue from the study.

All concomitant treatments, used from 12 weeks prior to first dose of IP until the End of Study visit must be reported on the eCRF.

All prior and concomitant blood and blood products received, iron chelating and antihypertensive therapies used from 24 weeks prior to first dose of IP until the End of Study visit must be reported on the eCRF.

8.1.2. Concomitant Medication for Anemia

Concurrent therapy with a new prescription medication related to the treatment of anemia during the course of the study must first be discussed with the Medical Monitor prior to administration, unless appropriate medical care necessitates that the medication should begin before the Medical Monitor can be consulted.

If treatment with hydroxyurea is required during the treatment period, the patient should be discontinued from treatment with study drug and enter the follow-up period.

8.1.3. Iron Chelation Therapy

Concurrent treatment with iron chelation therapy should be used as per product label and investigator practice prior to randomization.

8.1.4. RBC Transfusions

Concurrent treatment for anemia with blood transfusions is allowed, at the discretion of the Investigator, for low hemoglobin levels, symptoms associated with anemia (eg, hemodynamic or pulmonary compromise requiring treatment) or comorbidity.

For any RBC transfusions received during the study, collect hemoglobin values just prior to transfusion, the number of units transfused, the dates of transfusion. If available, the volume transfused in mL at each transfusion and hematocrit (Hct) of the transfused unit should be collected.

Each TD subject will have a "pre-transfusion hemoglobin threshold" for requiring transfusion during the study which will be determined based on transfusion history. Baseline pre-transfusion hemoglobin threshold will be the mean of all documented pre-transfusion hemoglobin values during the 24 weeks prior to Dose 1 Day 1. During treatment, if the pre-transfusion hemoglobin level is increased by ≥ 1 g/dL compared to the pre-transfusion hemoglobin threshold for that subject, transfusion should be delayed by a minimum of 7 days and/or the number of units transfused should be reduced by 1 or more RBC units.

Subjects may be transfused at the Investigator's discretion for symptoms related to anemia or other requirements (eg, infection).

8.2. Prohibited Concomitant Medications and Procedures

No concurrent treatments with any other investigational agent are allowed.

Treatment with hydroxyurea and anagrelide is not allowed during the Treatment Period (refer also to Section 8.1.2).

The use of hematopoietic growth factors will not be allowed during the Treatment Period of the study. However, any subject who requires hematopoietic growth factor treatment during the Treatment Period must be discontinued from the study, at which point, hematopoietic growth factor treatment is permitted.

Anticoagulant therapies and platelet aggregation inhibitors can be used. However, if these therapies are used due to a related AE that would qualify for Treatment Discontinuation as per Section 11, the subject should be discontinued from the study treatment. Any anticoagulant therapies use for prophylaxis, as well as aspirin and LMW heparin are allowed.

8.3. Required Concomitant Medications and Procedures

Not applicable.

9. STATISTICAL CONSIDERATIONS

9.1. Overview

The proposed Phase 3 study is double-blind, randomized, placebo- controlled, multicenter study to determine the efficacy and safety of luspatercept (ACE-536) plus BSC versus BSC in adults who require regular red blood cell transfusion due to β-thalassemia.

The design of the study, including the proposed targeted patient population, study endpoints, and statistical plan, is discussed below.

9.2. Study Population Definitions

Study populations to be analyzed are defined as follow:

Intent-to-treat (ITT): The ITT population will consist of all randomized subjects regardless of whether or not the subject received IP. All efficacy analyses will be conducted for the ITT population and will be analyzed based on randomization group.

Safety: The safety population will consist of all subjects who were randomized and received at least one dose of IP. Subjects will be included in the treatment group corresponding to the IP they actually received.

Statistical methods to handle missing data will be described in the SAP. The SAP will describe any predefined rules for including/excluding any subjects with data from any analyses (eg, time windows, visit by visit analysis, endpoint analysis, protocol violation).

9.3. Sample Size and Power Considerations

Based on data in the luspatercept Phase 2 (A536-04/A536-06) study the assumed targeted response rate for the primary endpoint is 40% in luspatercept group and 20% for the placebo group. A total sample size of 300 (200 in the luspatercept group, 100 in placebo group) will have 90% power to detect the difference between the luspatercept group and the placebo group with a 2-sided alpha of 0.05 and assumed 10% drop-out rate for each treatment group.

Randomization and Stratification

Subjects will be randomized to receive luspatercept or placebo at a 2:1 ratio. Randomization will be accomplished by an IRT to ensure timely registration and randomization. A stratified randomization schedule will be implemented. Randomization will be stratified by: Geographical regions[a]:

- North America and Europe
- Middle East and North Africa
- Asia-Pacific

[a] See Appendix E for list of countries within each region.

9.4. Background and Demographic Characteristics

Subjects' age, height, weight, and baseline characteristics will be summarized using descriptive statistics, while gender, race and other categorical variables will be provided using frequency

tabulations by dose cohort. Prior transfusion history will be summarized. Medical history data will be summarized using frequency tabulations by the Medical Dictionary for Regulatory Activities (MedDRA) system organ class and preferred term. Beta-thalassemia diagnoses as well as RBC transfusion dependence will be summarized using frequency tabulations.

9.5. Subject Disposition

Subject disposition (analysis population allocation, entered, discontinued, along with primary reason for discontinuation) will be summarized using frequency and percent for both treatment and follow-up phases. A summary of subjects enrolled by site will be provided. Protocol deviations will be summarized using frequency tabulations.

9.6. Efficacy Analysis

9.6.1. Primary Efficacy Analysis

The efficacy analysis will be performed on the ITT population. The primary efficacy analysis will be performed based on 24 weeks of data after all subjects have completed the double-blind 24-week treatment period phase or discontinue before reaching 24 weeks of double-blind treatment (for this study one month equal to 4 weeks is considered). A higher response rate in the luspatercept over placebo and a p-value < 0.05 will confirm the superiority of luspatercept in the efficacy.

The primary efficacy endpoint of this study is defined as subjects with ≥ 33% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 13 to Week 24 compared to the 12-week interval prior to randomization for luspatercept plus BSC versus placebo plus BSC.

For the early discontinued subjects, ie, who did not complete 24 weeks of double-blinded treatment period, the transfusion records will still be collected up to 48 weeks. All the transfusion records will be used to evaluate primary and secondary endpoints.

The first day to be used for statistical analysis will be defined as the date of the first dose. A transfusion received on Dose 1 Day 1 will be counted in the baseline transfusion burden.

The primary endpoint response rate is calculated using the number of responders divided by all subjects in the ITT population. The response rates of the subjects who randomized to luspatercept and the placebo will be calculated. Let P_1 denote the true response rate in the luspatercept group, and P_2 denote the true response rate in the placebo group. In the primary efficacy analysis, the statistical hypothesis is

$H_0 : P_1 = P_2$

$H_a : P_1 \neq P_2$

The number and percentage of subjects in the ITT population who achieve the response will be calculated for luspatercept and placebo. The difference in proportions between luspatercept and placebo will be calculated and the Cochran Mantel-Haenszel (CMH) chi-square test would be performed using randomization factors as strata, corresponding 95% confidence interval (CI)

associated with the test will be provided. A higher response rate in the luspatercept arm over placebo arm with a p-value < 0.05 will infer that luspatercept is significantly superior to placebo.

9.6.2. Secondary Efficacy Analysis

The analyses of secondary efficacy endpoints will be performed on the ITT population. The results will be presented by treatment groups. The statistical tests will be conducted to compare the treatment groups.

Gate-keeping methods will be used to control the overall Type 1 error rate for the secondary endpoints. After the result from the primary efficacy analysis in the ITT population shows statistical significance, the key secondary endpoint 1 will be tested next. The key secondary endpoint 2 will be tested only if the test results for both primary endpoint and the key secondary endpoint are significant. The key secondary endpoint 3 will be tested only if the test results for primary endpoint and the key secondary endpoints 1 and 2 are all significant. The testing procedure above will be implemented strictly in order to control the overall Type 1 error rate of 0.05 due to multiplicity.

The secondary endpoints will be measured at 24 weeks and 48 weeks from randomization and be statistically tested in a sequential order at $\alpha = 0.05$ level:

1. Proportion of subjects with hematological improvement, defined as ≥ 33% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 37 to Week 48.
2. Proportion of subjects with hematological improvement, defined as ≥ 50% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 13 to Week 24.
3. Proportion of subjects with hematological improvement, defined as ≥ 50% reduction from baseline in RBC transfusion burden with a reduction of at least 2 units from Week 37 to Week 48.
4. Mean change in transfusion burden (RBC units) from Week 13 to Week 24.

9.6.2.1. Proportion of Subjects with ≥ 33% Reduction from Baseline in RBC Transfusion Burden with a Reduction of at Least 2 Units from Week 37 to Week 48

Subjects will be analyzed as described in Section 9.6.1 for the primary endpoint.

9.6.2.2. Proportion of Subjects with ≥ 50% Reduction from Baseline in RBC Transfusion Burden with a Reduction of at Least 2 Units from Week 13 to Week 24

Subjects will be analyzed as described in Section 9.6.1 for the primary endpoint.

9.6.2.3. Proportion of Subjects with ≥ 50% Reduction from Baseline in RBC Transfusion Burden with a Reduction of at Least 2 Units from Week 37 to Week 48

Subjects will be analyzed as described in Section 9.6.1 for the primary endpoint

9.6.2.4. Mean Change in Transfusion Burden (RBC Units) from Week 13 to Week 24

The mean change in transfusion burden (RBC units) from baseline will be analyzed using ANCOVA method with stratification factor and baseline transfusion burden as covariates from Week 13 to Week 24. Corresponding 95% CI associated with the test will be provided.

9.6.3. Other Efficacy Analyses

Other efficacy analyses include:

1. Mean change in liver iron concentration (LIC, mg/g dw) by MRI
2. Mean change in mean daily dose of iron chelation therapy (ICT)
3. Change in serum ferritin
4. Mean change in total hip and lumbar spine bone mineral density (BMD) by DXA
5. Mean change in myocardial iron by MRI
6. Mean change in Quality of Life using TranQoL and SF36
7. Healthcare resource utilization
8. Proportion of subjects who are transfusion independent for ≥ 8 weeks during treatment
9. Duration of reduction in transfusion burden
10. Duration of transfusion independence
11. Time to erythroid response
12. Post-baseline transfusion events frequency versus placebo

In general, descriptive statistics will be provided and statistical tests will be applied if appropriate.

Kaplan-Meier methods will be used to analyze time to event secondary variables. All other secondary efficacy variables will be analyzed descriptively. Counts and percentages will be used to describe categorical secondary variables.

The statistical assumption for ANCOVA method will be validated, and log transformation may be applied as appropriate.

9.6.3.1. Mean Change in Liver Iron Concentration (LIC)

There are 3 scheduled LIC measurements for each subject: baseline, 24 weeks and 48 weeks. For subjects who discontinued before 48 weeks, LIC may also be measured at the time of discontinuation. The 48-week LIC change from baseline will be analyzed using an analysis of covariance (ANCOVA) with the randomization stratification factors and baseline LIC as covariates. For any subject, if the 48-week LIC measurement is not available, the last LIC measurement before 48 week will be used instead.

9.6.3.2. Mean Change in Mean Daily Dose of Iron Chelation Therapy (ICT)

For a subject, the baseline mean daily dose will be calculated using ICT dosage during the 12 weeks prior to first study drug treatment. The post baseline mean daily dose will be calculated using ICT dosage during the last 12 weeks of the 48 weeks double-blind Treatment Period or last

12 weeks of study treatment if discontinue early. The change in daily dose for each subject is calculated as the difference of post-baseline mean daily dose and baseline mean daily dose. ANCOVA will be used to compare the treatment difference between groups, with the stratification factor and baseline ICT value as covariates.

9.6.3.3. Mean Change in Serum Ferritin Level

For a subject, the baseline mean serum ferritin will be calculated during the 12 weeks prior to first study drug treatment. The post baseline mean serum ferritin will be calculated during the last 12 weeks of the 48 weeks double-blind Treatment Period or last 12 weeks of study treatment if discontinue early. The change is calculated as the difference of post-baseline mean serum ferritin and baseline mean serum ferritin. ANCOVA will be used to compare the treatment difference between groups, with the stratification factors and baseline serum ferritin value as covariates.

9.6.3.4. Bone Mineral Density Assessed by DXA Scan

For bone mineral density, the lumbar spine and total hip will be measured at baseline and at 48 weeks. Change from baseline lumbar spine score will be analyzed using ANCOVA method with randomization factor and baseline lumbar spine score as covariates. Change from baseline total hip score will be analyzed using ANCOVA method with randomization factors and baseline total hip score as covariates. The analysis will be done on the population that have at least 2 measurements.

9.6.3.5. Mean Change in Myocardial Iron by MRI

There are 2 scheduled myocardial iron measurements for each subject: baseline and 48 weeks. For subjects who discontinued before 48 weeks, myocardial iron will be measured at the time of discontinuation. The 48-week myocardial iron change from baseline will be analyzed using an analysis of covariance (ANCOVA) with the randomization stratification factors and baseline myocardial iron as covariates. For any subject, if the 48-week myocardial iron measurement is not available, the last myocardial iron measurement before 48 week will be used instead.

9.6.3.6. Change in Quality of Life using TranQol and SF-36

SF-36: The SF-36 Version 2.0 is a self-administered instrument consisting of 8 multi-item scales that assess 8 health domains: Physical functioning, Role-Physical, Bodily Pain, General Health, Vitality, Social functioning, Role-Emotional, and Mental Health. Two overall summary scores (Physical Component and Mental Component) can also be obtained. Summary statistics (N, mean, median, standard deviation [SD], range, skewness, kurtosis) for the Physical functioning, Role – Physical, General Health, Vitality, Social Functioning domain scores, and the Physical Component summary score, as well as change from baseline in these scores, will be assessed at Week 12, 24, 36 and 48 and then every 12 weeks during the Long-term Treatment Period. Summary statistics will be calculated for the total sample and for each treatment group. Scoring for the SF-36 and methods to address missing values within an assessment (ie, scores missing for individual items) will follow directions provided by the instrument developers.

Mixed-model analyses will examine the effect of luspatercept treatment on change in the SF-36 domain scores from baseline to 48 weeks. The models will include the SF-36 change scores from

baseline as the outcome (in separate analyses), and time, treatment group assignment, stratification factors, and a time-by-treatment group assignment term in the model. The mixed-model analysis allows for subjects to contribute different numbers of data points to analysis. Therefore, there will be no score estimation for missing assessments. We will assume an unstructured correlation structure for the analyses of these data. The test of the significance of the interaction terms in these models will provide a test of differences across time for the two treatment arms. If time-by-treatment group assignment term is not significant, we will exclude the interaction term from the model, and the final model will include the SF-36 change scores from baseline as the outcome, and time, treatment group assignment, and stratification factor. In this model, the significance of overall treatment effect will be tested by the treatment group assignment term.

TranQol: Chronically transfused patients with thalassemia major have a unique set of concerns that are not adequately addressed by generic quality of life tools. The TranQol is a tool specific to this population. The TranQol is a new disease-specific quality of life instrument developed for adult Beta-thalassemia patients.

Summary statistics (N, mean, median, SD, range, skewness, kurtosis) for scores from the pre-specified domains of emotional and the School / Career domains and the total score will be calculated at each administration time point (baseline, weeks 12, 24, 36 and 48 and then every 12 weeks during the Long-term Treatment Period) for the total sample and each treatment group. Summary statistics will also be calculated for change from baseline in each score at each administration time point for the total sample and each treatment group. Scoring for the TranQoL and methods to address missing values within an assessment (ie, scores missing for individual items) will follow directions provided by the instrument developers.

Mixed-model analyses will examine the effect of luspatercept treatment on change in the TranQoL total score and domain scores from baseline to 48 weeks. The models will include the TranQoL change scores from baseline as the outcome (in separate analyses), and time, treatment group assignment, stratification factors, and a time-by-treatment group assignment term in the model. The mixed-model analysis allows for subjects to contribute different numbers of data points to analysis. Therefore, there will be no score estimation for missing assessments. We will assume an exchangeable correlation structure for the analyses of these data. The test of the significance of the interaction terms in these models will provide a test of differences across time for the two treatment arms. If time-by-treatment group assignment term is not significant, we will exclude the interaction term from the model, and the final model will include the TranQoL change scores from baseline as the outcome, and time, treatment group assignment, and stratification factor. In this model, the significance of overall treatment effect will be tested by the treatment group assignment term.

9.6.3.7. Healthcare Resource Utilization

Characterization of medical resource utilization among subjects treated with luspatercept plus BSC as compared to subjects receiving placebo plus BSC treatment. Health care resource utilization includes an aggregation of hospitalizations, prior concomitant therapies and surgeries, as well as RBC transfusion utilization. Details of the analysis will be provided in the SAP.

9.6.3.8. Duration of Transfusion Burden Reduction

The duration of the response will be calculated for each subject who achieves a response. The general algorithms used to calculate the duration of response are as follows:

First Day of Response = the first day of showing response. Last Day of Response = last day of showing response. Date of Last Assessment = either the last visit date for subjects still on drug or the date of discontinuation for subjects who discontinued from the treatment.

The duration of response will be calculated as follows, depending on whether or not the response ends before the Date of Last Assessment:

- For subjects who response does not continue to the end of the treatment period, the duration of response is not censored, and is calculated as
  Response Duration = Last Day of Response – First Day of Response +1.
- For subjects who continue to respond at the end of the treatment period, the end day of the response is censored and duration of the response is calculated as
  Response duration = Date of Last Assessment – First Day of Response +1

  The longest duration will be used if there are multiple intervals of transfusion burden reduction, and the sum of the durations will be summarized if applicable.
  Only subjects who achieve response will be included in the analysis. For each treatment group, the Kaplan–Meier method will be used to estimate the median duration of response and to calculate the 95% CI for the median duration of response. The Kaplan-Meier plots of the time to end of response will also be provided.

9.6.3.9. Time to Response

The time to the first response will be calculated for subjects who achieved a response as follows:

Time to Response = First Day of Response – Date of First Study Drug +1

The analysis of time to response will be based on the data collected during the double-blind phase. The time to the first response will be summarized using descriptive statistics by treatment groups.

9.6.3.10. Transfusion Independence

Transfusion independence is defined as the absence of any transfusion during any consecutive "rolling" 8-week time interval within the treatment period, ie, Days 1 to 56, Days 2 to 57 and so on. Subjects discontinued from double-blind treatment for lack of therapeutic effect or with less than 56 days of assessment during the double-blind treatment period will be counted as non-responders. The number and percent of subjects who achieve transfusion independence (defined as at least 8 weeks transfusion-free) will be compared between the two treatment groups. The Cochran Mantel-Haenszel (CMH) test with stratification factor as strata would be performed with randomization factors as strata, corresponding 95% CI associated with the test will be provided.

9.6.3.11. Postbaseline Transfusion Events Frequency Versus Placebo

The postbaseline transfusion event frequency will be analyzed using negative binomial regression with randomization stratification factor and baseline transfusion frequency in the regression model.

Annualized mean change from baseline number of transfusion events will be summarized by treatment groups

9.7. Safety Analysis

All safety analyses will be performed on the safety population. Full details will be included in the SAP. Planned data presentations and analyses include the following:

- Adverse events will be coded using MedDRA. Adverse event listings will include the verbatim term and the MedDRA preferred term. Treatment-emergent adverse events will be summarized by worst severity grade, system organ class, and preferred term. Treatment-emergent adverse events leading to death or to discontinuation from treatment, TEAEs classified as NCI CTCAE (version 4.0) all grades and grade 3/4 TEAEs, related to investigational product, and serious TEAEs will be summarized separately.
- Clinical laboratory results will be summarized descriptively by treatment group. Clinically significant hematologic and non-hematologic laboratory abnormalities will be listed and summarized according to the NCI CTCAE by treatment group.
- Vital sign measurements, including body weight, will be listed for each subject at each visit. Descriptive statistics for vital signs, both observed values and changes from baseline, will be summarized by treatment group.

Cross tabulations will be provided to summarize frequencies of abnormalities.

Descriptive statistics will be provided for vital sign and body weight data.

By-subject listings will be provided for all relevant safety data. Graphical displays and figures will be provided where useful to assist in the interpretation of results.

9.8. Interim Analysis

No Interim Analysis is planned.

9.9. Timing of Analyses

9.9.1. Final Analysis

9.9.1.1. Clinical Study Report for Marketing Authorization Application

A clinical study report (CSR) for a marketing authorization application (MAA) will include safety and efficacy parameters at the time of the final analysis when all subjects completed 48 weeks of a double-blind Treatment Period or discontinued before reaching 48 weeks. With this cut-off date and upon the data base lock the study will be unblinded.

The efficacy analyses included in the CSR for MAA will be conducted on the primary endpoint, secondary endpoints, and safety endpoints.

9.9.1.2. Final Clinical Study Report

The final CSR will include efficacy and safety data at the time of the End of the Trial.(ie, when all subjects initially assigned to luspatercept in the double-blind Treatment Period have completed the total treatment duration of 5 years from the subject's Dose 1 Day 1 in the treatment period of this ACE-536-B-THAL-001 study or have discontinued earlier and completed the 156-week Post-treatment Follow-up Period, whichever occurred later.)

The final efficacy analyses will be conducted on primary endpoint, secondary endpoints and safety endpoints.

9.10. Other Topics

CCI [redacted]

9.10.2. Data Monitoring Committee (DMC)

The independent DMC will be comprised of expert(s) in β-thalassemia not involved in ACE-536-B-THAL-001 Protocol, an Independent Cardiologist, an Independent Statistician, and may include additional ad hoc members. Representatives of the Sponsor will be attending the blinded part of the DMC meetings. The Sponsor will not have access to the unblinded data.

During the course of the study, the DMC will review the unblinded safety data regularly as well as safety and efficacy data in accordance with the guidelines for the preplanned analyses. An independent third party will prepare the reports of aggregate data summaries and individual subject data listings, as appropriate, to the DMC members for each scheduled meeting.

The DMC responsibilities, authorities, and procedures will be detailed in the DMC charter, which will be endorsed by the DMC prior to the first data review meeting.

Operational details for the DMC will be detailed in the DMC charter.

9.10.3. Steering Committee

A Steering Committee will be established by charter for this study. The Steering Committee will be comprised of Study Investigators, Sponsor representatives, and may include additional ad hoc

members. The Steering Committee will review blinded data. The Steering Committee will serve in an advisory capacity to the Sponsor. The Steering Committee will advise and recommend to the Sponsor:

- Changes to the protocol or conduct of the study based upon emerging clinical or scientific data from this and/or other studies.
- Procedures to ensure the safety of subjects and integrity of study data.
- Procedures to meet the overall goals and objectives of the study.

The Steering Committee responsibilities, authorities, and procedures will be detailed in the Steering Committee charter, which will be endorsed by the Steering Committee prior to the first data review meeting.

Operational details for the Steering Committee will be detailed in a separate Steering Committee charter.

Note: The Steering Committee is separate from the DMC.

CCI [redacted]

10. ADVERSE EVENTS

10.1. Monitoring, Recording and Reporting of Adverse Events

An AE is any noxious, unintended, or untoward medical occurrence that may appear or worsen in a subject during the course of a study. It may be a new intercurrent illness, a worsening concomitant illness, an injury, or any concomitant impairment of the subject's health, including laboratory test values (as specified by the criteria in Section 10.3), regardless of etiology. Any worsening (ie, any clinically significant adverse change in the frequency or intensity of a pre-existing condition) should be considered an AE. A diagnosis or syndrome should be recorded on the AE page of the eCRF rather than the individual signs or symptoms of the diagnosis or syndrome.

Abuse, withdrawal, sensitivity or toxicity to an investigational product should be reported as an AE. Overdose, accidental or intentional, whether or not it is associated with an AE should be reported on the overdose eCRF. (See Section 7.2.1.3 for the definition of overdose.) Any sequela of an accidental or intentional overdose of an investigational product should be reported as an AE on the AE eCRF. If the sequela of an overdose is a serious adverse event (SAE), then the sequela must be reported on an SAE report form and on the AE eCRF. The overdose resulting in the SAE should be identified as the cause of the event on the SAE report form and eCRF but should not be reported as an SAE itself.

In the event of overdose, the subject should be monitored as appropriate and should receive supportive measures as necessary. There is no known specific antidote for luspatercept overdose. Actual treatment should depend on the severity of the clinical situation and the judgment and experience of the treating physician.

All subjects will be monitored for AEs during the study. Assessments may include monitoring of any or all of the following parameters: the subject's clinical symptoms, laboratory, pathological, radiological or surgical findings, physical examination findings, or findings from other tests and/or procedures.

All AEs regardless of causality will be recorded by the Investigator from the time the subject signs informed consent until 9 weeks after the last dose of IP as well as those SAEs made known to the Investigator at any time thereafter that are suspected of being related to IP. In addition, AEs that are suspected to be related to the IP as well as new malignancies or pre-malignancies regardless of causality (see Section 10.5.2) will be recorded by the Investigator until 156 weeks after the last dose of IP. Information will be collected as described in the Table of Events, Table 3 and Section 6.6. AEs and SAEs will be recorded on the AE page of the eCRF and in the subject's source documents. All SAEs must be reported to Celgene Drug Safety within 24 hours of the Investigator's knowledge of the event by facsimile, or other appropriate method, using the SAE Report Form, or approved equivalent form.

10.2. Evaluation of Adverse Events

A qualified Investigator will evaluate all adverse events as to:

10.2.1. Seriousness

An SAE is any AE occurring at any dose that:

- Results in death;
- Is life-threatening (ie, in the opinion of the Investigator, the subject is at immediate risk of death from the AE);
- Requires inpatient hospitalization or prolongation of existing hospitalization (hospitalization is defined as an inpatient admission, regardless of length of stay);
- Results in persistent or significant disability/incapacity (a substantial disruption of the subject's ability to conduct normal life functions);
- Is a congenital anomaly/birth defect;
- Constitutes an important medical event.

Important medical events are defined as those occurrences that may not be immediately life-threatening or result in death, hospitalization, or disability, but may jeopardize the subject or require medical or surgical intervention to prevent one of the other outcomes listed above. Medical and scientific judgment should be exercised in deciding whether such an AE should be considered serious.

Events **not considered** to be SAEs are hospitalizations for:

- a standard procedure for protocol therapy administration. However, hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as an SAE.
- routine treatment or monitoring of the studied indication not associated with any deterioration in condition.
- the administration of blood or platelet transfusion as routine treatment of studied indication. However, hospitalization or prolonged hospitalization for a complication of such transfusion remains a reportable SAE.
- a procedure for protocol/disease-related investigations (eg, surgery, scans, endoscopy, sampling for laboratory tests, bone marrow sampling). However, hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable SAE.
- hospitalization or prolongation of hospitalization for technical, practical, or social reasons, in absence of an AE.
- a procedure that is planned (ie, planned prior to start of treatment on study); must be documented in the source document and the eCRF. Hospitalization or prolonged hospitalization for a complication remains a reportable SAE.

- an elective treatment of or an elective procedure for a pre-existing condition, unrelated to the studied indication, that has not worsened from baseline.
- emergency outpatient treatment or observation that does not result in admission, unless fulfilling other seriousness criteria above.

If an AE is considered serious, both the AE page/screen of the eCRF and the SAE Report Form must be completed.

For each SAE, the Investigator will provide information on severity, start and stop dates, relationship to the IP, action taken regarding the IP, and outcome.

10.2.2. Severity/ Intensity

For both AEs and SAEs, the Investigator must assess the severity/ intensity of the event.

The severity/intensity of AEs will be graded based upon the subject's symptoms according to the current active minor version of the Common Terminology Criteria for Adverse Events (CTCAE, Version 4.0);
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40

AEs that are not defined in CTCAE should be evaluated for severity/intensity according to the following scale:

- Grade 1 = Mild – transient or mild discomfort; no limitation in activity; no medical intervention/therapy required
- Grade 2 = Moderate – mild to moderate limitation in activity, some assistance may be needed; no or minimal medical intervention/therapy required
- Grade 3 = Severe – marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization is possible
- Grade 4 = Life-threatening – extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable
- Grade 5 = Death - the event results in death

The term "severe" is often used to describe the intensity of a specific event (as in mild, moderate or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This criterion is *not* the same as "serious" which is based on subject/event *outcome* or *action* criteria associated with events that pose a threat to a subject's life or functioning.

Seriousness, not severity, serves as a guide for defining regulatory obligations.

10.2.3. Causality

The Investigator must determine the relationship between the administration of the IP and the occurrence of an AE/SAE as Not Suspected or Suspected as defined below:

Not suspected: a causal relationship of the adverse event to IP administration is **unlikely or remote**, or other medications, therapeutic

interventions, or underlying conditions provide a sufficient explanation for the observed event.

Suspected: there is a **reasonable possibility** that the administration of IP caused the adverse event. 'Reasonable possibility' means there is evidence to suggest a causal relationship between the IP and the adverse event.

Causality should be assessed and provided for every AE/SAE based on currently available information. Causality is to be reassessed and provided as additional information becomes available.

If an event is assessed as suspected of being related to a comparator, ancillary or additional IP that has not been manufactured or provided by Celgene, please provide the name of the manufacturer when reporting the event.

10.2.4. Duration

For both AEs and SAEs, the Investigator will provide a record of the start and stop dates of the event.

10.2.5. Action Taken

The Investigator will report the action taken with IP as a result of an AE or SAE, as applicable (eg, discontinuation, interruption, or dose reduction of IP, as appropriate) and report if concomitant and/or additional treatments were given for the event.

10.2.6. Outcome

The Investigator will report the outcome of the event for both AEs and SAEs.

All SAEs that have not resolved upon discontinuation of the subject's participation in the study must be followed until recovered (returned to baseline), recovered with sequelae, or death (due to the SAE).

10.3. Abnormal Laboratory Values

An abnormal laboratory value is considered to be an AE if the abnormality:

- results in discontinuation from the study;
- requires treatment, modification/ interruption of IP dose, or any other therapeutic intervention; or
- is judged to be of significant clinical importance, eg, one that indicates a new disease process and/or organ toxicity, or is an exacerbation or worsening of an existing condition.

Regardless of severity grade, only laboratory abnormalities that fulfill a seriousness criterion need to be documented as a serious adverse event.

If a laboratory abnormality is one component of a diagnosis or syndrome, then only the diagnosis or syndrome should be recorded on the AE page/screen of the eCRF. If the abnormality was not

a part of a diagnosis or syndrome, then the laboratory abnormality should be recorded as the AE. If possible, the laboratory abnormality should be recorded as a medical term and not simply as an abnormal laboratory result (eg, record thrombocytopenia rather than decreased platelets).

10.4. Pregnancy

All pregnancies or suspected pregnancies occurring in either a female subject of childbearing potential or partner of childbearing potential of a male subject are immediately reportable events.

10.4.1. Females of Childbearing Potential

Females of childbearing potential will be advised to avoid becoming pregnant during the study and for 12 weeks after the last dose of IP. Pregnancies and suspected pregnancies (including elevated βhCG or positive pregnancy test in a female subject of childbearing potential regardless of disease state) occurring while the subject is on IP, or within 12 weeks of the subject's last dose of IP, are considered immediately reportable events. Investigational product is to be discontinued immediately. The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to Celgene Drug Safety immediately by email, phone or facsimile, or other appropriate method, using the Pregnancy Initial Report Form, or approved equivalent form.

The female subject may be referred to an obstetrician-gynecologist or another appropriate healthcare professional for further evaluation.

The Investigator will follow the female subject until completion of the pregnancy, and must notify Celgene Drug Safety immediately about the outcome of the pregnancy (either normal or abnormal outcome) using the Pregnancy Follow-up Report Form, or approved equivalent form.

If the outcome of the pregnancy was abnormal (eg, spontaneous abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE to Celgene Drug Safety by facsimile, or other appropriate method, within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form.

All neonatal deaths that occur within 28 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 28 days that the Investigator suspects is related to the in utero exposure to the IP should also be reported to Celgene Drug Safety by facsimile, or other appropriate method, within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form.

10.4.2. Male Subjects

If a female partner of a male subject taking IP becomes pregnant, the male subject taking IP should notify the Investigator, and the pregnant female partner should be advised to call their healthcare provider immediately.

Males will be advised to use a latex condom during any sexual contact with a FCBP prior to starting investigational product and continue for 12 weeks following the last dose of IP, even if he has undergone a successful vasectomy.

10.5. Reporting of Serious Adverse Events

Any AE that meets any criterion for an SAE requires the completion of an SAE Report Form in addition to being recorded on the AE page/screen of the eCRF. All SAEs must be reported to Celgene Drug Safety within 24 hours of the Investigator's knowledge of the event by facsimile, or other appropriate method (eg, via email), using the SAE Report Form, or approved equivalent form. This instruction pertains to initial SAE reports as well as any follow-up reports.

The Investigator is required to ensure that the data on these forms is accurate and consistent. This requirement applies to all SAEs (regardless of relationship to IP) that occur during the study (from the time the subject signs informed consent until 9 weeks after the last dose of IP) or any SAE made known to the Investigator at any time thereafter that are suspected of being related to IP until the End of Study. Serious adverse events occurring prior to treatment (after signing the ICF) will be captured.

The SAE report should provide a detailed description of the SAE and include a concise summary of hospital records and other relevant documents. If a subject died and an autopsy has been performed, copies of the autopsy report and death certificate are to be sent to Celgene Drug Safety as soon as these become available. Any follow-up data should be detailed in a subsequent SAE Report Form, or approved equivalent form, and sent to Celgene Drug Safety.

Where required by local legislation, the Investigator is responsible for informing the Institutional Review Board/Ethics Committee (IRB/EC) of the SAE and providing them with all relevant initial and follow-up information about the event. The Investigator must keep copies of all SAE information on file including correspondence with Celgene and the IRB/EC.

10.5.1. Safety Queries

Queries pertaining to SAEs will be communicated from Celgene Drug Safety to the site via facsimile or electronic mail. The response time is expected to be no more than five (5) business days. Urgent queries (eg, missing causality assessment) may be handled by phone.

10.5.2. Malignancy and Pre-malignancy Reporting

The occurrence of a new malignancy or pre-malignant lesion will be monitored as an event of interest and should be included as part of the assessment of adverse events throughout the course of the study. Investigators are to report the development of any new malignancy or pre-malignant lesion as a serious adverse event, regardless of causal relationship to IP, occurring at any time for the duration of the study, from the time of signing the ICF up to and including 156 weeks of follow-up.

Events of new malignancy or pre-malignant lesions (excluding benign tumors or benign neoplasia) are to be reported to Celgene Drug Safety within 24 hours of the Investigator's knowledge of the event by facsimile, or other appropriate method, using the SAE Report Form, or approved equivalent form and must be considered an "Important Medical Event" even if no other serious criteria apply; these events must also be documented in the appropriate page(s) of the CRF and subject's source documents. Documentation of the diagnosed malignancy must be provided at the time of reporting as a serious adverse event (eg, any confirmatory histology or cytology results, x-rays, CT scans, etc.).

Malignancies or cancerous tumors are lesions capable of invading into adjacent tissues, and may be capable of spreading to distant tissues. A benign tumor has none of those properties.

Malignancy or cancer is characterized by anaplasia, invasiveness, and metastasis.

Pre-malignant or precancerous lesions refer to a state of disordered morphology of cells that is associated with an increased risk of cancer. If left untreated, these conditions may lead to cancer. Such conditions are usually either dysplasia or benign neoplasia (and the dividing line between those is sometimes blurry). Sometimes the term "precancer" is used to describe carcinoma in situ, which is a noninvasive cancer that has not progressed to an aggressive, invasive stage. Not all carcinoma in situ will progress to invasive disease.

Pre-malignant lesions are morphologically atypical tissue which appears abnormal under microscopic examination, and in which cancer is more likely to occur than in its apparently normal counterpart.

10.6. Expedited Reporting of Adverse Events

For the purpose of regulatory reporting, Celgene Drug Safety will determine the expectedness of events suspected of being related to luspatercept based on the Investigator Brochure.

In the United States, all suspected unexpected serious adverse reactions (SUSARs) will be reported in an expedited manner in accordance with 21 CFR 312.32.

For countries within the European Economic Area (EEA), Celgene or its authorized representative will report in an expedited manner to Regulatory Authorities and Ethics Committees concerned, SUSARs in accordance with Directive 2001/20/EC and the Detailed Guidance on collection, verification and presentation of adverse reaction reports arising from clinical trials on investigational products for human use (ENTR/CT3) and also in accordance with country-specific requirements.

In addition, any report of hematologic malignancy regardless of causality in the luspatercept arm, will be reported to the regulatory authorities in an expedited manner, if requested.

Celgene or its authorized representative shall notify the Investigator of the following information:

- Any AE suspected of being related to the use of IP in this study or in other studies that is both serious and unexpected (ie, SUSAR);
- Any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Where required by local legislation, the Investigator shall notify his/her IRB/EC promptly of these new serious and unexpected AE(s) or significant risks to subjects.

The Investigator must keep copies of all pertinent safety information on file including correspondence with Celgene and the IRB/EC. (See Section 14.3 for record retention information).

Celgene Drug Safety Contact Information:

For Celgene Drug Safety contact information, please refer to the Serious Adverse Event Report Form Completion Guidelines or to the Pregnancy Report Form Completion Guidelines.

11. DISCONTINUATIONS

11.1. Treatment Discontinuation

Subjects will have a treatment discontinuation visit at the time of study drug discontinuation. All subjects who received at least one dose of the study drug will be followed for 156 weeks post-treatment.

The following events are considered sufficient reasons for discontinuing a subject from the investigational product(s):

- Adverse Event(s):
  - Any serious AE assessed as related to luspatercept
  - Any AE ≥ Grade 3 assessed to be related to luspatercept if the event causes a treatment delay of 15 weeks or longer from the prior administered dose, including hypersensitivity to luspatercept.
  - If a subject experiences > 2 Dose Reductions due to related AE
- Any new malignancy
- Withdrawal of consent - An indication that a study participant has removed itself from the study treatment
- Death
- Lost to follow-up - the loss or lack of continuation of a subject to follow-up
- Pregnancy
- If dose is delayed for more than 15 weeks or longer from the prior dose administered dose due to AE, including case of elective surgery/hospitalization
- Other: Different than the one(s) previously specified or mentioned

The reason for discontinuation of treatment should be recorded in the eCRF and in the source documents.

The decision to discontinue a subject from treatment remains the responsibility of the treating physician, which will not be delayed or refused by the Sponsor. However, prior to discontinuing a subject, the Investigator may contact the Medical Monitor and forward appropriate supporting documents for review and discussion.

11.2. End of Study

The following events **are** considered sufficient reasons for discontinuing a subject from the study:

- Screen failure
- Completed study per protocol
- Withdrawal of consent - An indication that a study participant has removed itself from the study

- Death
- Lost to follow-up - The loss or lack of continuation of a subject to follow-up
- Other: Different than the one(s) previously specified or mentioned

The following events **may be** considered sufficient reasons for discontinuing a subject from the study:

- Adverse events(s) - Any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. NOTE: For further information, see the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting. [Modified from ICH E2A] Synonyms: side effect, adverse experience. See also serious adverse event, serious adverse experience

The reason for End of Study should be recorded in the eCRF and in the source documents.

12. EMERGENCY PROCEDURES

12.1. Emergency Contact

In emergency situations, the Investigator should contact the responsible Clinical Research Physician/Medical Monitor or designee by telephone at the number(s) listed on the Emergency Contact Information page of the protocol (after title page).

In the unlikely event that the Clinical Research Physician/Medical Monitor or designee cannot be reached, please contact the global Emergency Call Center by telephone at the number listed on the Emergency Contact Information page of the protocol (after title page). This global Emergency Call Center is available 24 hours a day and 7 days a week. The representatives are responsible for obtaining your call-back information and contacting the on-call Celgene/contract research organization Medical Monitor, who will then contact you promptly.

Note: The back-up 24-hour global emergency contact call center should only be used if you are not able to reach the Clinical Research Physician(s) or Medical Monitor or designee for emergency calls.

12.2. Emergency Identification of Investigational Products (Emergency Unblinding)

The blind must not be broken during the course of the study **unless** in the opinion of the Investigator, it is absolutely necessary to safely treat the subject. If it is medically imperative to know what IP the subject is receiving, IP should be temporarily discontinued if, in the opinion of the Investigator, continuing IP can negatively affect the outcome of the subject's treatment.

The decision to break the blind in emergency situations remains the responsibility of the treating physician, which will not be delayed or refused by the Sponsor. However, the Investigator may contact the Medical Monitor prior to breaking the blind to discuss unblinding, mainly in the interest of the subject.

The Investigator should ensure that the code is broken only in accordance with the protocol. The Investigator should promptly notify the Medical Monitor of the emergency unblinding and the reason for breaking the blind, which should be clearly documented by the Investigator in the subject's source documentation.

Emergency unblinding should only be performed by the Investigator through the IRT by using an emergency unblinding personal identification number (PIN), and the Investigator should call IRT for unblended dose information.

13. REGULATORY CONSIDERATIONS

13.1. Good Clinical Practice

The procedures set out in this study protocol pertaining to the conduct, evaluation, and documentation of this study are designed to ensure that Celgene, its authorized representative, and Investigator abide by Good Clinical Practice (GCP), as described in International Conference on Harmonisation (ICH) Guideline E6 and in accordance with the general ethical principles outlined in the Declaration of Helsinki. The study will receive approval from an IRB/EC prior to commencement. The Investigator will conduct all aspects of this study in accordance with applicable national, state, and local laws of the pertinent regulatory authorities.

13.2. Investigator Responsibilities

Investigator responsibilities are set out in the ICH Guideline for Good Clinical Practice and in the local regulations. Celgene staff or an authorized representative will evaluate and approve all Investigators who in turn will select their staff.

The Investigator should ensure that all persons assisting with the study are adequately informed about the protocol, amendments, study treatments, as well as study-related duties and functions, including obligations of confidentiality of Celgene information. The Investigator should maintain a list of Sub-investigators and other appropriately qualified persons to whom he or she has delegated significant study-related duties.

The Investigator is responsible for keeping a record of all subjects who sign an informed consent form (ICF) and are screened for entry into the study. Subjects who fail screening must have the reason(s) recorded in the subject's source documents.

The Investigator, or a designated member of the Investigator's staff, must be available during monitoring visits to review data, resolve queries and allow direct access to subject records (eg, medical records, office charts, hospital charts, and study-related charts) for source data verification. The Investigator must ensure timely and accurate completion of eCRFs and queries.

The information contained in the protocol and amendments (with the exception of the information provided by Celgene on public registry websites) is considered Celgene confidential information. Only information that is previously disclosed by Celgene on a public registry website may be freely disclosed by the Investigator or its institution, or as outlined in the Clinical Trial Agreement. Celgene protocol, amendment and IB information is not to be made publicly available (for example on the Investigator's or their institution's website) without express written approval from Celgene. Information proposed for posting on the Investigator's or their institution's website must be submitted to Celgene for review and approval, providing at least 5 business days for review.

At the time results of this study are made available to the public, Celgene will provide Investigators with a summary of the results that is written for the lay person. The Investigator is responsible for sharing these results with the subject and/or their caregiver as agreed by the subject.

13.3. Subject Information and Informed Consent

The Investigator must obtain informed consent of a subject and/or a subject's legal representative prior to any study related procedures.

Documentation that informed consent occurred prior to the study subject's entry into the study and of the informed consent process should be recorded in the study subject's source documents including the date. The original ICF signed and dated by the study subject and by the person consenting the study subject prior to the study subject's entry into the study, must be maintained in the Investigator's study files and a copy given to the study subject. In addition, if a protocol is amended and it impacts on the content of the informed consent, the ICF must be revised. Study subjects participating in the study when the amended protocol is implemented must be re-consented with the revised version of the ICF. The revised ICF signed and dated by the study subject and by the person consenting the study subject must be maintained in the Investigator's study files and a copy given to the study subject.

13.4. Confidentiality

Celgene affirms the subject's right to protection against invasion of privacy and to be in compliance with ICH and other local regulations (whichever is most stringent). Celgene requires the Investigator to permit Celgene's representatives and, when necessary, representatives from regulatory authorities, to review and/or copy any medical records relevant to the study in accordance with local laws.

Should direct access to medical records require a waiver or authorization separate from the subject's signed ICF, it is the responsibility of the Investigator to obtain such permission in writing from the appropriate individual.

13.5. Protocol Amendments

Any amendment to this protocol must be approved by the Celgene Clinical Research Physician/Medical Monitor. Amendments will be submitted to the IRB/EC for written approval. Written approval must be obtained before implementation of the amended version occurs. The written signed approval from the IRB/EC should specifically reference the Investigator name, protocol number, study title and amendment number(s) that is applicable. Amendments that are administrative in nature do not require IRB/IEC approval but will be submitted to the IRB/IEC for information purposes.

13.6. Institutional Review Board/Independent Ethics Committee Review and Approval

Before the start of the study, the study protocol, ICF, and any other appropriate documents will be submitted to the IRB/EC with a cover letter or a form listing the documents submitted, their dates of issue, and the site (or region or area of jurisdiction, as applicable) for which approval is sought. If applicable, the documents will also be submitted to the authorities in accordance with local legal requirements.

IP can only be supplied to an Investigator by Celgene or its authorized representative after documentation on all ethical and legal requirements for starting the study has been received by

Celgene or its authorized representative. This documentation must also include a list of the members of the IRB/EC and their occupation and qualifications. If the IRB/EC will not disclose the names, occupations and qualifications of the committee members, it should be asked to issue a statement confirming that the composition of the committee is in accordance with GCP. For example, the IRB General Assurance Number may be accepted as a substitute for this list. Formal approval by the IRB/EC should mention the protocol title, number, amendment number (if applicable), study site (or region or area of jurisdiction, as applicable), and any other documents reviewed. It must mention the date on which the decision was made and must be officially signed by a committee member. Before the first subject is enrolled in the study, all ethical and legal requirements must be met.

The IRB/EC and, if applicable, the authorities, must be informed of all subsequent protocol amendments in accordance with local legal requirements. Amendments must be evaluated to determine whether formal approval must be sought and whether the ICF should also be revised.

The Investigator must keep a record of all communication with the IRB/EC and, if applicable, between a Coordinating Investigator and the IRB/EC. This statement also applies to any communication between the Investigator (or Coordinating Investigator, if applicable) and regulatory authorities.

Any advertisements used to recruit subjects for the study must be reviewed by Celgene and the IRB/EC prior to use.

13.7. Ongoing Information for Institutional Review Board/ Ethics Committee

If required by legislation or the IRB/EC, the Investigator must submit to the IRB/EC:

- Information on serious or unexpected adverse events as soon as possible;
- Periodic reports on the progress of the study;
- Deviations from the protocol or anything that may involve added risk to subjects.

13.8. Termination of the Study

Celgene reserves the right to terminate this study prematurely at any time for reasonable medical or administrative reasons. Any premature discontinuation will be appropriately documented according to local requirements (eg, IRB/EC, regulatory authorities, etc).

The Sponsor may consider closing this trial when data supporting key endpoints and objectives of the study have been analyzed. In the case where there are subjects still being administered the investigational product, and it is the opinion of the Investigator(s) that these subjects continue to receive benefit from treatment, the Sponsor may choose to initiate an open-label, roll-over or extension study under a separate protocol to allow these subjects continued access to luspatercept following their participation in this study until the drug is commercially available.

In addition, the Investigator or Celgene has the right to discontinue a single site at any time during the study for medical or administrative reasons such as:

- Unsatisfactory enrollment;
- GCP noncompliance;
- Inaccurate or incomplete data collection;
- Falsification of records;
- Failure to adhere to the study protocol.

14. DATA HANDLING AND RECORDKEEPING

14.1. Data/Documents

The Investigator must ensure that the records and documents pertaining to the conduct of the study and the distribution of the investigational product are complete, accurate, filed and retained. Examples of source documents include: hospital records; clinic and office charts; laboratory notes; memoranda; subject's diaries or evaluation checklists; dispensing records; recorded data from automated instruments; copies or transcriptions certified after verification as being accurate copies; microfiche; x-ray film and reports; and records kept at the pharmacy, and the laboratories, as well as copies of eCRFs or CD-ROM.

14.2. Data Management

Data will be collected via eCRF and entered into the clinical database per Celgene standard operating procedures (SOPs). This data will be electronically verified through use of programmed edit checks specified by the clinical team. Discrepancies in the data will be brought to the attention of the clinical team, and investigational site personnel, if necessary. Resolutions to these issues will be reflected in the database. An audit trail within the system will track all changes made to the data.

14.3. Record Retention

Essential documents must be retained by the Investigator according to the period of time outlined in the clinical trial agreement. The Investigator must retain these documents for the time period described above or according to local laws or requirements, whichever is longer. Essential documents include, but are not limited to, the following:

- Signed ICFs for all subjects;
- Subject identification code list, screening log (if applicable), and enrollment log;
- Record of all communications between the Investigator and the IRB/EC;
- Composition of the IRB/EC;
- Record of all communications between the Investigator, Celgene, and their authorized representative(s);
- List of Sub-investigators and other appropriately qualified persons to whom the Investigator has delegated significant study-related duties, together with their roles in the study, curriculum vitae, and their signatures;
- Copies of CRFs (if paper) and of documentation of corrections for all subjects;
- IP accountability records;
- Record of any body fluids or tissue samples retained;
- All other source documents (subject records, hospital records, laboratory records, etc.);

- All other documents as listed in Section 8 of the ICH consolidated guideline on GCP (Essential Documents for the Conduct of a Clinical Trial).

The Investigator must notify Celgene if he/she wishes to assign the essential documents to someone else, remove them to another location or is unable to retain them for a specified period. The Investigator must obtain approval in writing from Celgene prior to destruction of any records. If the Investigator is unable to meet this obligation, the Investigator must ask Celgene for permission to make alternative arrangements. Details of these arrangements should be documented.

All study documents should be made available if required by relevant health authorities. Investigator or institution should take measures to prevent accidental or premature destruction of these documents.

15. QUALITY CONTROL AND QUALITY ASSURANCE

All aspects of the study will be carefully monitored by Celgene or its authorized representative for compliance with applicable government regulations with respect to current GCP and SOPs.

15.1. Study Monitoring and Source Data Verification

Celgene ensures that appropriate monitoring procedures are performed before, during and after the study. All aspects of the study are reviewed with the Investigator and the staff at a study initiation visit and/or at an Investigators' Meeting. Prior to enrolling subjects into the study, a Celgene representative will review the protocol, eCRFs, procedures for obtaining informed consent, record keeping, and reporting of AEs/SAEs with the Investigator. Monitoring will include on-site visits with the Investigator and his/her staff as well as any appropriate communications by mail, email, fax, or telephone. During monitoring visits, the facilities, investigational product storage area, eCRFs, subject's source documents, and all other study documentation will be inspected/reviewed by the Celgene representative in accordance with the Study Monitoring Plan.

Accuracy will be checked by performing source data verification that is a direct comparison of the entries made onto the eCRFs against the appropriate source documentation. Any resulting discrepancies will be reviewed with the Investigator and/or his/her staff. Any necessary corrections will be made directly to the eCRFs or via queries by the Investigator and/or his/her staff. Monitoring procedures require that informed consents, adherence to inclusion/exclusion criteria and documentation of SAEs and their proper recording be verified. Additional monitoring activities may be outlined in a study-specific monitoring plan.

15.2. Audits and Inspections

In addition to the routine monitoring procedures, a Good Clinical Practice Quality Assurance unit exists within Celgene. Representatives of this unit will conduct audits of clinical research activities in accordance with Celgene appropriate SOPs to evaluate compliance with Good Clinical Practice guidelines and regulations.

The Investigator is required to permit direct access to the facilities where the study took place, source documents, eCRFs and applicable supporting records of study subject participation for audits and inspections by IRB/ECs, regulatory authorities (eg, the Food and Drug Administration [FDA], European Medicines Agency [EMA], Health Canada) and company authorized representatives. The Investigator should make every effort to be available for the audits and/or inspections. If the Investigator is contacted by any regulatory authority regarding an inspection, he/she should contact Celgene immediately.

15.3. Product Quality Complaint

A Product Quality Complaint (PQC) is any written, electronic, or oral communication that alleges deficiencies related to the identity, quality, durability, reliability, safety, effectiveness, purity, or performance of any drug product manufactured by or on behalf of Celgene Corporation after it is released for distribution. PQCs may reduce the usability of the product for its intended function or affect performance of the product and therefore pose a significant risk to the subject. Examples of PQCs include (but are not limited to): mixed product, mislabeling, lack of effect,

seal/packaging breach, product missing/short/overage, contamination, suspected falsified, tampered, diverted or stolen material, and general product/packaging damage. If you become aware of a suspected PQC, you are obligated to report the issue immediately. You can do so by emailing PPD PPD or by contacting CCI

16. PUBLICATIONS

As described in Section 13.2, all protocol- and amendment-related information, with the exception of the information provided by Celgene on public registry websites, is considered Celgene confidential information and is not to be used in any publications. Celgene protocol-related information proposed for use in a publication must be submitted to Celgene for review and approval, and should not be utilized in a publication without express written approval from Celgene, or as described in the Clinical Trial Agreement.

Celgene will ensure Celgene-sponsored studies are considered for publication in the scientific literature in a peer-reviewed journal, irrespective of the results. At a minimum, this applies to results from all Phase 3 clinical studies, and any other study results of significant medical importance. This also includes results relating to investigational medicines whose development programs have been discontinued.

Study results may also be presented at one or more medical congresses, and may be used for scientific exchange and teaching purposes. Additionally, this study and its results may be submitted for inclusion in all appropriate health authority study registries, as well as publication on health authority study registry websites, as required by local health authority regulations.

CCI [redacted]

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18. APPENDICES

Appendix A: Table of Abbreviations

Table 6: Abbreviations and Specialist Terms

Abbreviation or Specialist Term	Explanation
ActRIIB	Activin receptor type IIB
ADA	Antidrug antibody
AE	Adverse event
ALT	Alanine aminotransferase (SGPT)
ANC	Absolute neutrophil count
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase (SGOT)
ATG	Anti-thymocite globulin
AUC	Area under the curve
β-hCG	β-subunit of human chorionic gonadotropin
BMD	Bone mineral density
BSC	Best supportive care
BUN	Blood urea nitrogen
CI	Confidence interval
CFR	Code of Federal Regulations
C_{max}	Maximum plasma concentration of drug
CMH	Cochran Mantel-Haenszel
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
DFO	Deferoxamine
DFP	Deferiprone
DFX	Deferasirox
DMC	Data Monitoring Committee
dw	Dry weight
DXA	Dual energy x-ray absorptiometry
EC	Ethics Committee

Table 6: Abbreviations and Specialist Terms (Continued)

Abbreviation or Specialist Term	Explanation
ECD	Extracellular domain
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
EEA	European Economic Area
EMA	European Medicines Agency
EPO	Erythropoietin
ESA	Erythropoiesis-stimulating agent
EU	European Union
FCBP	Female of childbearing potential
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HbF	Fetal hemoglobin
Hb	Hemoglobin
Hct	Hematocrit
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HRU	Healthcare Resource Utilization
HSCT	Hematopoietic stem cell transplantation
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Conference on Harmonisation
ICT	Iron chelation therapy
ID	Identification
IND	Investigational New Drug
IP	Investigational Product
IRB	Institutional Review Board
IRT	Integrated Response Technology
ITT	Intent-to-treat (subjects)

Table 6: Abbreviations and Specialist Terms (Continued)

Abbreviation or Specialist Term	Explanation
IWRS	Integrated Web Response System
LDH	Lactic dehydrogenase
LIC	Liver iron concentration
LMW	Low-molecular-weight
LLN	Lower limit of the normal
LVEF	Left ventricular ejection fraction
MAA	Marketing authorization application
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
ms	milliseconds
MUGA	Multi Gated Acquisition Scan
NCI	National Cancer Institute
NTD	Non-transfusion dependent
NYHA	New York Heart Association
PK	Pharmacokinetics
PQC	Product Quality Complaint
QoL	Quality of Life
RBC	Red blood cell count
RDW	Red blood cell distribution width
ROS	Reactive oxygen species
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous(ly)
SD	Standard deviation
SF	Short Form
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase

Table 6: Abbreviations and Specialist Terms (Continued)

Abbreviation or Specialist Term	Explanation
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
TD	Transfusion dependent
$t_{1/2}$	Half-life
t_{max}	Mean time to C_{max}
ULN	Upper limit of normal
TEAE	Treatment-emergent adverse event
TGF-β	Transforming growth factor-β
TRV	Tricuspid regurgitant velocity
WBC	White blood cell
WFI	Water for injection

Appendix B: ECOG Performance Status Scale

The Eastern Cooperative Oncology Group (ECOG) scale (Oken, 1982) is used to assess a patient's quality of life in an evaluation by a health professional of the daily activities and how the activities are affected by the disease of the patient.

ECOG Performance Status Scale

Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, eg, light housework, office work.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

Appendix C: National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0

Currently active minor version of NCI CTCAE, Version 4.0:

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

http://www.oncology.tv/SymptomManagement/NationalCancerInstituteUpdatesCTCAEtov403.aspx

Appendix D: New York Heart Association - Classification of Heart Failure

New York Heart Association - Classification of Heart Failure

Class	Symptoms
Class 1	No limitation of activities. No symptoms from ordinary activities
Class 2	Mild limitation of activity. Comfortable with rest or mild exertion
Class 3	Marked limitation of activity and be comfortable only at rest
Class 4	Complete rest, confined to bed or chair; any physical activity brings on discomfort and symptoms occur at rest

Appendix E: List with Countries within each Region

Table 7: Countries Within Each Region

Countries included in each Geographical region may include, but are not limited to:

North America & Europe	Middle East & North Africa	Asia-Pacific
Bulgaria	Algeria	Australia
Canada	Egypt	Hong Kong
France	Israel	Malaysia
Germany	Lebanon	Taiwan
Greece	Morocco	Thailand
Italy	Oman	
Hungary	Saudi Arabia	
United Kingdom	Tunisia	
Unites States	Turkey	
	United Arab Emirates	

Celgene Signing Page

This is a representation of an electronic record that was signed electronically in Livelink. This page is the manifestation of the electronic signature(s) used in compliance with the organizations electronic signature policies and procedures.

UserName: PPD
Title: PPD
Date: Thursday, 13 December 2018, 05:13 PM Eastern Daylight Time
Meaning: Approved, no changes necessary.
==========

1. JUSTIFICATION FOR AMENDMENT

Significant changes included in this amendment are summarized below. These changes were based on feedback obtained from the Health Authorities, and internal discussions.

1.1. Dose Modifications: Dose Delay, Dose Reduction and Discontinuation Guidelines

CCI [redacted] additional criteria for dose adjustment have been introduced, ie:

- Dose delay for condition of worsening of anemia, as described in Table 5 and Dose discontinuation, if hematological malignancy is confirmed
- Dose delay for condition of Grade ≥ 3 leukopenia, neutropenia and/or thrombocytopenia, and dose discontinuation if hematological malignancy is confirmed

Revised Sections: Section 7.2, Section 7.2.1.2, Table 5

1.2. Other Minor Clarification and Corrections

The amendment also includes several other minor clarifications and corrections:

1.2.1. Correction of Typos Identified in the Document:

- Section 3.2. Study Duration for Subjects: End of Study for each individual subject: Instead of "96-week Post Treatment Follow-up" it should read "156-week Post Treatment Follow-up"
- Section 9.9.1.1. Clinical Study Report for Marketing Authorization Application : clarified that the data cut-off date for the CSR for MAA as described in the Protocol Section 9.9.1.1 will be at the time "when all subjects completed 48 weeks of a double-blind Treatment Period or discontinued before reaching 48 weeks". The following clarifier has been removed "The CSR for MAA will include TEAEs reported for 9 weeks post last dose." Since the Clinical data base will remain open to collect any TEAEs reported for 9 weeks post last dose and related AEs until the End of Study (as already clarified in Section 5, Table 3, as well as Section 10.1. Thus, in the scenario if the last subjects discontinue treatment within the 9 weeks of the predefined data base cut-off date for the CSR for the MAA all available TEAEs will be added to an updated report to the Agency, upon request.
- Section 3.1.3. Long-term Treatment Period: to align the duration of the Long-Term Treatment Period and the timing of unblinding with the information in Section 9.9.1.1, the following clarification has been updated: *Subjects who continue to receive treatment with luspatercept or placebo when the study is unblinded (approximately ~~a maximum~~ 48 weeks after the first dose of the last subject and upon the database lock) may opt to continue receiving luspatercept in the Open-label Phase or to discontinue treatment and enter the Post-Treatment Follow-up Period*

- Protocol Summary: corrected typo “no transfusion-free period > 35 days”, instead of “ > 45 days".
- Sections 6.2, Section 6.3, Section 6.4, Section 6.5, Section 6.6.1: “abnormal ultrasound” it should read “abdominal ultrasound”
- Section 15.3 Product Quality Compliant was added to clarify the process for communicating any PQC issue to Celgene Customer Care Center.
- In the Table of Events, Table 3, clarifications have been added to the serum chemistry, urinalysis and serum ferritin that these assessment are evaluated by local laboratory in the Open-label Phase of the study.
- Additional editorial revisions or minor clarifications

1. JUSTIFICATION FOR AMENDMENT

Significant changes included in this amendment are summarized below. These changes were based on feedback obtained from the Health Authorities, Ethic Committees, Investigators, Steering Committee (SC), Data Monitoring Committee (DMC) and internal discussions.

CCI [redacted]

1.2. Study Design

1.2.1. Placebo subjects: Allow all subjects, regardless of the initial treatment to which they have been randomized in the double-blind Treatment Period, to receive active treatment in the Open-label Phase.

Rationale:

In the last quarter of 2015 and during the course of 2016, CCI [redacted] as well as Investigators participating in the ACE-536-B-THAL-001 study, have commented that it is unethical towards subjects participating in this study, to not allow all subjects to receive active drug in the Open-label Phase of the study. Based on feedback received from investigators, the biggest challenge for enrollment in this study will be subject's skepticism of being enrolled in the placebo arm, and thus subjects perception that there is no benefit for them in participating in the study if they are in the placebo group. In December 2015, Celgene met with the DMC and SC for this study to share the feedback received. Both DMC and SC committees were in agreement for the current protocol to be modified to allow all subjects regardless of the initial treatment they have been assigned to in the double-blind Treatment Period to have the opportunity to receive active treatment in an Open-label Phase. On 16 Dec

2015, Celgene signed a formal letter indicating Celgene commitment in amending the protocol to allow all subjects to receive activate treatment in an Open-label Phase. In June 2016, Celgene further met with the study SC and presented the proposed modified study design: the modified study design laid down in this protocol Amendment 1 has been discussed and was agreed upon by the SC at the 10 Jun 2016 SC meeting.

Revised Sections: Section 3.1.3, 3.1.4, 3.1.5, 3.2, 3.3, 6.4, Table 3, Figures 4 and 5, and the Protocol Summary.

1.2.2. Inclusion of the Open-label Extension study as part of the current study, ie, the Open-label Phase and not a separate study

Rationale:

Based on internal discussions, Celgene considered for practical reasons to incorporate the Open-label Extension study, initially mentioned as a separate study, as part of this ongoing study. This approach has been considered as more practical from subject and site perspective, and will avoid moving subjects from one study to a new one, as well as avoid multiple administrative procedures at the site level, such as closing and opening of the studies. This item has been reviewed and was agreed upon by the study SC following the 10 Jun 2016 meeting.

Revised Sections: Section 3.1.3, 3.1.4, 3.1.5, 3.2, 3.3, 6.4, Table 3, Figures 4 and 5.

1.2.3. Open-label Phase design

Rationale:

The initiation and duration of the Open-label Phase as outlined in this Amendment 1 is: *Open-label Phase will begin after the study unblinding. The commencement of this Open-label Phase will be determined by the enrollment rate and by the availability of primary analysis data that justify the use of luspatercept in an Open-label Phase, which will be reviewed by the independent external DMC. After DMC review of safety and efficacy, DMC will determine if the use of luspatercept in subjects previously randomized to receive placebo in this Open-label phase is safe and recommended, and if subjects already on luspatercept can continue to be treated at their current dose level (best supportive care is allowed). In the Open-label Phase, subjects may receive luspatercept until all subjects initially assigned to luspatercept in the double-blind Treatment Period, complete the total treatment duration of 5 years from subjects' Dose 1 Day 1 in the treatment period of this ACE-536-B-THAL-001 study or discontinue early.*

For subjects initially assigned to luspatercept:

- It has been clarified that subjects initially assigned to luspatercept should not have discontinued the double-blind phase, in order to be eligible for the Open-label Phase.

  →Thus, subjects that have been already assigned to active treatment and who are experiencing benefit of being on active treatment can continue in the Open-label Phase.

For subjects initially assigned to placebo:

- It has been clarified that subject initially assigned to placebo can enter the Open-label Phase even if they have discontinued the double-blind phase, but were compliant with the

protocol 48 weeks post Dose 1 Day 1, and continue their participation in the Post-treatment Follow-up Period until the time of unblinding.

→The compliance with the protocol 48 weeks post Dose 1 Day 1 is needed to allow evaluation of the primary and key secondary endpoints. The visit schedule in the Post-treatment Follow-up Period is a very light schedule approximately every 24 weeks, so that the visit burden to the subject is decreased at a minimum up to the time of unblinding.

- It has been clarified that placebo subjects who discontinued treatment and continued their participation in the Post-treatment Follow-up Period until the time of unblinding will have the opportunity to enter the Open-label Phase, if they **still fulfill the main eligibility criteria**, prior to receiving their first dose of luspatercept.

  →The main safety/comorbidity eligibility criteria has been considered as a condition to enter the Open-label Phase.

Revised Sections: Section 3.1.4

1.2.4. Extended the Post-treatment Follow-up Period (post last dose follow-up) from 9 weeks to 156 weeks.

CCI

[redacted]

CCI [redacted]

Revised Sections: Section 3.1.5, 3.2, 3.3, 11.1, and the Protocol Summary.

1.2.5. Overall Study Design figure and Subject Management Figure

Rationale:

To reflect the change in the study design, the Study Design Figure 4 has been updated. In addition to further provide practical guidance to the sites on subject flow management within the study from Screening/Run-in up to the End of Study, a Subject Management Decision Tree has been created.

Revised Sections: Figure 4 and Figure 5

1.2.6. Inclusion of malignancy and pre-malignancy reporting in Post-treatment Follow-up

Rationale:

CCI [redacted]

As described in the protocol: The occurrence of a new malignancy or pre-malignant lesion will be monitored and collected as an event of interest and should be included as part of the assessment of adverse events throughout the course of the study. Investigators are to report the development of any new malignancy or pre-malignant lesion as a serious adverse event, regardless of causal relationship to IP (study drug[s] or control), occurring at any time for the duration of the study, from the time of signing the ICF up to and including 156 weeks of follow-up (refer to Section 10 for additional information).

This item has been reviewed with the study SC following the 10 Jun 2016 meeting.

Revised Sections: Section 5 (Table 3, Table of Events), Sections 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.6.1, 6.7, 10.1, and 10.5.2.

1.2.7. Implementing subject diary for transfusion received outside of the regular transfusion center

Rationale:

Implementation of a diary in order to capture any transfusions occurring outside the investigation site, when there is not an established plan in place. The intention is to increase the accuracy of the transfusions and hemoglobin data record. This item has been reviewed and was agreed upon by the study SC at the 10 Jun 2016 meeting.

Revised Sections: Section 5 (Table 3, Table of Events), Sections 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.6.1.

1.2.8. Provide flexibility between the Randomization call and Dose 1 Day 1 from 24 hours to 3 days

Rationale:

For practical reasons at the sites per feedback received from the Investigators. This item has been reviewed and was agreed upon by the study SC following the 10 June 2016 meeting.

Revised Section: Section 3.1.2

1.2.9. Allow the transfusion to be administered at Dose 1 Day 1

Rationale:

Per Investigators' feedback and request - for practical reasons to avoid bringing the subjects to the site for additional visits.

If a transfusion occurs on Dose 1 Day 1, the first dose of luspatercept should not be given within 1 hour before the start of the transfusion, or 2 hours after the end of the transfusion is implemented to avoid confounding factors in the event of a patient reaction to either a transfusion or to the first dose of study medication.

Revised Section: Section 3.1.2

1.3. Eligibility Criteria

1.3.1. Inclusion criterion 4 –clarification to β-thalassemia diagnosis in regards to the mutation profile and changed to Inclusion criterion 4 instead of Exclusion criterion 4

Rationale:

For clarity, based on Investigators' feedback. This item has been reviewed and was agreed upon by the study SC following the 10 Jun 2016 meeting.

Revised Sections: Sections 4.2 and Section 4.3

1.3.2. Inclusion criteria 7 and 8 – contraception language: updated language to list acceptable contraception methods

Rationale:

CCI [redacted] The protocol was amended to include details of effective contraception for women of childbearing potential. The protocol should be consistent with regards to the terms "adequate contraception" and "effective contraception." This item has been reviewed and was agreed upon by the study SC following the 10 Jun 2016 meeting.

Revised Section: Section 4.2

1.3.3. Exclusion criterion 5 – clarified definition of active hepatitis B and hepatitis C

Rationale:

Based on requests received from Investigators to clarify this eligibility criterion.

Revised Section: Section 4.3

1.3.4. Exclusion criterion 7 – clarified chronic anticoagulant therapy uses in the context of exclusion criterion

Rationale:

It has been clarified that the anticoagulant therapies used for prophylaxis for surgery or high risk procedures as well as low-molecular-weight (LMW) heparin for superficial venous thrombosis and chronic aspirin are allowed. This change has been implemented based on requests received from Investigators to clarify this eligibility criterion.

Revised Section: Section 4.3

1.3.5. Exclusion criterion 9 – clarified insulin-dependent diabetes, ie, chronic treatment with insulin criterion to specify: "*Poorly controlled diabetes mellitus within 24 weeks prior to randomization as defined by short term (eg, hyperosmolar or ketoacidotic crisis) and/or history of diabetic cardiovascular complications (eg, stroke or myocardial infarction).*"

Rationale:

Clarification as per Investigators request. Excluding subjects prone to complex concomitant medical issues that may interfere with the study participation and the safety data irrespective of insulin dependency. This item has been reviewed and was agreed upon by the study SC following the 10 June 2016 meeting.

Revised Section: Section 4.3

1.3.6. Exclusion criterion 17 a) – simplified the cirrhosis evidence in the exclusion criterion

Rationale:

Based on feedback received from Investigators, to allow subjects with minor liver fibrosis. Celgene does not anticipate that luspatercept may worsen the fibrotic process in the liver, and the metabolism/elimination of luspatercept does not depend on liver function. This allows enrollment of subjects with stable, asymptomatic, and uncomplicated liver fibrosis. This item has been reviewed and was agreed upon by the study SC following the 10 Jun 2016 meeting.

Revised Section: Section 4.3

1.3.7. Exclusion criterion 17 c) –added toxicity Grade 3 as per Common Terminology Criteria for Adverse Events (CTCAE) as a reference for clinically significant lung disease and pulmonary fibrosis

Rationale:

Based on requests received from Investigators to clarify this eligibility criterion.

Note: Grade 3 CTCAE refers to:

- clinically significant pulmonary hypertension is considered severe symptoms associated with hypoxemia; right-sided heart failure; oxygen indicated;

- clinically significant pulmonary fibrosis is considered severe hypoxemia; evidence of right-sided heart failure; radiographic pulmonary fibrosis >50 - 75%.

This item has been reviewed and was agreed upon by the study SC following the 10 Jun 2016 meeting.

Revised Section: Section 4.3

1.3.8. Exclusion criterion 19 – clarified adrenal insufficiency exclusion criterion to specify "*Systemic glucocorticoids ≤ 12 weeks prior to randomization (physiologic replacement therapy for adrenal insufficiency is allowed)*"

Rationale:

Based on requests received from Investigators to clarify this eligibility criterion. This item has been reviewed with the study SC following the 10 Jun 2016 meeting.

Revised Section: Section 4.3

1.3.9. Exclusion criterion 22 –Examples of immunosuppressant agents were provided for clarity

Rationale:

Examples of immunosuppressant agents were provided in the exclusion criterion for clarity.

Revised Section: Section 4.3

1.3.10. Exclusion criterion 23 – exclusion criterion on malignancy were created

Rationale:

CCI [redacted]

Revised Section: Section 4.3

1.4. Assessments and Procedures

1.4.1. Eastern Cooperative Oncology Group (ECOG) measurement during study treatment was removed; ECOG measurement at eligibility was not changed

Rationale:

Per feedback received from investigators: ECOG evaluation could be very subjective since it depends on the investigator assessing the subject; could be a concern if the investigator/sub-investigator changes from baseline to post-baseline. In addition, performance status is already captured during the trial via the Quality of Life (QoL) instruments. This item has been reviewed with the study SC following the 10 Jun 2016 meeting.

Revised Sections: Section 5, Table 3; Sections 6.1, 6.2, and 6.4, 6.5, 6.6, 6.7.

1.4.2. Malignancy and Pre-malignancy Reporting implemented in the Table of Events as well as Procedure Section of the protocol

Rationale:

To address the inclusion of the malignancy and pre-malignancy reporting into the study design (see Rationale as described above in Section 1.2.6 of the Justification for Amendment)

Revised Sections: Section 5, Table 3; Sections 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, and 6.7.

1.4.3. Subject Transfusion Diary implemented in the Table of Events as well as Procedure Section of the protocol

Rationale:

To address the inclusion of the subject diary into the study design (see Rationale as described above in Section 1.2.7 of the Justification for Amendment)

Revised Sections: Section 5, Table 3; Sections 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, and 6.6.1.

1.4.4. LVEF – MRI technique added as an option, MUGA technique clarified not applicable for Germany

Rationale:

MRI technique added as an option – clarification added, as per feedback received from sites, when measuring T2* (already required measurement per protocol), the LVEF can be taken out from the same MRI acquisition.

MUGA technique clarified N/A for Germany - [redacted].

Revised Sections: Section 5, Table 3; Sections 6.1, 6.2, 6.3, Protocol Summary.

1.4.5. Hematology Panel – clarification added reticulocytes and erythroblast to be measured by local laboratory

Rationale:

Due to stability of the reticulocytes, shipments from some sites to a central laboratory are not possible, thus Celgene decided to require reticulocyte count measurement by local lab.

Erythroblast measurement by an automated machine is a preferable outcome for erythroblast reading. Due to a different technique used in the central laboratory, a clarification for local measurement is implemented in the protocol.

Revised Sections: Section 5, Table 3; Sections 6.1, 6.2, 6.3, 6.4, 6.5, 6.6.

1.4.6. Spleen measurement by abdominal ultrasound implemented as technique for spleen measurement

Rationale:

Based on feasibility some sites do not use MRI for measuring the spleen. The Investigators requested the protocol be changed to allow spleen measurement by ultrasound, which is the standard practice at the sites.

Revised Sections: Section 5, Table 3; Sections 6.1, 6.2, 6.3, 6.4, 6.5, 6.6.1.

1.4.7. DXA measurement removed from Week 24

Rationale:

After additional analysis of Phase 2 data, significant changes in bone metabolisms at week 24 was not observed. In addition, as per feedback received from investigators Week 24 is too short of a time to highlight significant changes in bone metabolism.

Revised Sections: Section 5, Table 3; Sections 6.1, 6.2, 6.3, 6.4, 6.5, 6.6.1.

CCI [redacted]

1.4.9. Visit window increased: to +/- 5 days in Long-term Treatment Period and +/- 10 days for magnetic resonance imaging (MRI) and dual energy x-ray absorptiometry (DXA) assessments

Rationale:

As per Investigator feedback, to allow more flexibility in scheduling assessments and patient convenience.

Revised Sections: Section 5, Table 3; Section 6, 6.3.

1.4.10. Open-label Phase visit and assessments reflected in the Table of Events and Procedure Section

Rationale:

To reflect modification to the study design, as described above (Section 1.2.3).

Revised Sections: Section 5, Table 3; Sections 6, 6.4, 6.4.1, and 6.9

1.4.11. Post-treatment Follow-up: Follow-Up weeks 24, 48, 72, 96, 120, 144 and 156 visits and assessments reflected in the Table of Events and Procedure Section

Rationale:

To reflect modification to the study design, as described above (Section 1.2.4, 1.2.5).

Revised Sections: Section 5, Table 3; Sections 6.6 and 6.7.

1.4.12. Post-treatment Follow-up assessments on Follow-up week 6, replaced with Follow-up week 9

Rationale:

Schedule adjusted to capture the assessments outcome at the end of the period 5 times mean half-life of the IP.

Revised Sections: Section 5, Table 3 and Section 6.6.

1.4.13. Collection of ICTs, Post-Medications, transfusions, HRU and AEs changed from being recorded on an ongoing basis until end of study to until 9 weeks post last dose

Rationale:

The reporting of these items is kept up to 9 weeks post last dose, considering the 5 times half-life of the drug. After this period it is considered that the drug will not have effect. For AE, reporting of related AE until End of Study is kept unchanged.

Revised Sections: Section 5, Table 3; Sections 6.6, 6.6.1 and 6.7.

1.4.14. Clarified PK and ADA collection in Long-term Treatment Period and at Treatment Discontinuation

Rationale:

To protect the blinding it has been clarified that ADA results will not be required to decide the need of ADA monitoring prior to the study unblinding, with details described as following.

Long-term Treatment Period: PK and ADA samples will be collected every 6 doses for up to 2 years from Dose 1 Day 1 of the double blinded treatment period

Open Label Phase: For placebo-cross-over subjects, only if positive at unblinding, continue every 6 doses up to 2 years from Dose 1 Day 1 of the double blinded treatment period. For subjects continuing treatment with luspatercept, only if positive at unblinding, every 6 doses up to 2 years from Dose 1 Day 1 of the double blinded treatment period.

Post-treatment Follow-up: Before unblinding, ADA monitoring (every 24 weeks) at post-treatment follow-up visits will continue for up to two years from Dose 1 Day 1 of double-blind Treatment Period. After unblinding, ADA monitoring (every 24 weeks) at all post-treatment follow-up visits will continue only if subjects' last available ADA is positive and they have not reached the 2-year maximum limit for ADA monitoring.

Revised Sections: Section 5, Table 3; Section 6.9

1.5. Treatment Administration and IP

1.5.1. Clarification to IP Reconstitution and IP stability

Rationale:

As per investigator feedback, the wording on IP reconstitution has been clarified.

Additional information became available on drug stability since the original protocol version of 25 Aug 2015, thus the stability of the reconstituted product has been updated from 6 hours to 10 hours.

Revised Section: Section 7.1.

1.5.2. Clarification on placebo supply

Rationale:

Placebo supply language in the protocol has been adjusted to comply at a global scale with local country requirements for placebo supply.

Revised Sections: Sections 7.1, 7.4.1, and Protocol Summary.

1.5.3. Maximum volume per injection site increase to 1.2 ml.

Rationale:

In Phase 2 studies, the maximum volume per injection site is 1.2 mL, with acceptable subject comport. Therefore, maximum volume per injection site in the current protocol is increased from 1 ml to 1.2 ml to decrease the number of injections. .

Revised Sections: Sections 7.2.

1.5.4. Maximum total dose introduced up to 120 mg

CCI [redacted]

[redacted]

Revised Sections: Sections 7.2, 7.2.1.1 and 3.1.2.

1.5.5. Study Drug Administration: Clarification that the increase hemoglobin (Hb) ≤ 2.0 g/dL is to be compared to the pre-dose Hb of Day 1 of the previous treatment dose cycle (previous wording “at Day 21 from last dose”)

Rationale:

Clarification for consistency in the wording with protocol Table 5.

Revised Section: Section 7.2.

1.5.6. Study Drug Administration: Clarification that the pre-treatment/ pre-transfusion Hb value should be <11.5 g/dL for dose administration, the notion "not influenced by transfusion (ie, ≥14 days post- transfusion)" deleted from the paragraph.

Rationale:

As per received feedback from Investigator, the Sponsor updated this paragraph to avoid confusions in regards to the Hb level at the day of the dosing and the time of the transfusions. As clarified at the Investigator meetings held in 2016, if the pre-treatment/ pre-transfusion Hb value at the date of the dosing is < 11.5 g/dL the dose can be administered. The Hb influence by a transfusion is define in Table 5. Dose Delay, Dose Reduction and Discontinuation Guidelines.

Revised Section: Section 7.2

1.5.7. Study Drug Administration: introduced criteria for WBC threshold as additional criteria for dose administration

Rationale:

CCI [redacted] additional criteria for dose administration has been introduced, ie WBC absolute value at the day of the dosing, should note be more than 3 times WBC measured at baseline.

Revised Section: Section 7.2.

1.5.8. Revised Section: Section 7.2 Dose Titration – clarified that dose titration if transfusion reduction is ≤ 50% (previous < 50%) over at least two dose cycles (~6 weeks) is allowed.

Rationale:

Clarification as per investigator feedback.

Revised Section: Section 7.2.1.1.

1.5.9. Dose Titration – clarified that dose titration special request may be allowed for subjects who have been dose reduced once, but lost the response to treatment.

Rationale:

It has been clarified that "*After safety and efficacy data review, the sponsor may allow dose titration following special requests, such as, but not limited to, subjects who have been dose reduced once, but lost the response to treatment*".

Revised Section: Section 7.2.1.1.

1.5.10. Clarified that Dose Delay for AE unrelated to the study drug, as per discretion of the Investigator are allowed

Rationale:

Clarification added in order to allow dose delays for safety reasons other than related AE to avoid protocol deviations, as per received feedback from investigators.

Revised Section: Section 7.2.1.2

1.5.11. Dose Delay, Dose Reduction and Discontinuation Guidelines: introduced threshold for Dose adjustments in respect to the WBC counts compared to baseline WBC counts

Rationale:

CCI [redacted] additional criteria for dose adjustment has been introduced, ie fold increase of the WBC absolute value at the day of the dosing, compare to the WBC measured at baseline.

Revised Section: Section 7.2.1.2, Table 5

1.6. Concomitant Medications

1.6.1. Removed references for medication leading to treatment discontinuation from sections other than Section 8.2

Rationale:

Medications that will trigger treatment discontinuation are described in the prohibited medications section (Section 8.2). For clarity, and consistency references to such medications have been removed from other sections.

Revised Sections: Section 8 and Section 8.1.1.

1.6.2. Anticoagulant therapies used during the study treatment are allowed as long as the medications are not used due to a related adverse event (AE) that would qualify for treatment discontinuation

Rationale:

Clarified that the use of anticoagulant therapies will trigger treatment discontinuation only if such therapies are used due to a related AE that would qualify for treatment discontinuation as per Section 11 of the protocol.

Revised Section: Section 8.2.

1.6.3. Anagrelide added as prohibited medication

Rationale:

This class of drug-cAMP phosphodiesterase inhibitor, predisposes to prolonged QT interval and requires close monitoring with ECG and ECHO. In the β-thalassemia population this poses an additional risk of further potential cardiac morbidity [BC Cancer Agency Cancer Drug Manual for Anagrelide p 2/7(1st Nov 2015)].

Revised Section: Section 8.2.

1.7. Statistical Section

1.7.1. Clarification that transfusion received on Dose 1 Day 1 will be counted in the baseline transfusion burden.

Rationale:

Clarification added to reflect the modification that a transfusion can be administered on Dose 1 Day 1. This approach has been presented and reviewed with the SC at the 10 Jun 2016 SC meeting.

Revised Sections: Section 9.6.1

1.7.2. Clarification added to the Other efficacy analyses section that log transformation may be applied as appropriate.

Rationale:

The statistical assumption for an analysis of covariance (ANCOVA) method will be validated, which will allow the statistical methodology to be more complete.

Revised Section: Section 9.6.3

1.7.3. Clarification to the duration of transfusion burden reduction

Rationale:

Modification added for clarity that "the longest duration will be used if there are multiple intervals and the sum of the durations might be summarized if applicable." This modification provides options if multiple intervals occur.

Revised Section: Section 9.6.3.8

1.7.4. Bone Mineral Density – clarified that the analysis will be done on the population that have at least 2 measurements.

Rationale:

Clarification added to reflect removal of week 24 DXA assessment.

Revised Section: Section 9.6.3.4.

1.7.5. Clarification to the regression method used in the postbaseline transfusion events frequency versus placebo

Rationale:

Clarified that the regression method to be used in analyzing the post baseline transfusion event frequency will be negative binomial regression, instead of Poisson regression. This will provide statistically better options to estimate the variance.

Revised Section: Section 9.6.3.11

1.7.6. Interim Analysis (IA) – IA has been removed from the protocol

Rationale:

The interim analysis has been originally implemented in the study to manage the timeline risk of the study if subject enrollment was very slow. As of 4 April 2017, 281 subjects have been randomized in the study, projected closure of randomizations is currently targeted in Jun 2017, which is approximately one year earlier than originally planned end of randomization . Current maintenance of high enrollment rates have diminished the purpose of the interim analysis, prompting to the decision to not perform the analysis and proceed to final analysis as per the study SAP. Subject safety is monitor on ongoing basis by independent DMC, thus removing the IA from the protocol, will not impact the already described regular safety monitoring in the protocol.

Revised Sections: Section 9.8 and Protocol Summary.

1.7.7. Final Analysis – clarified CSR for MAA and Final CSR

Rationale:

With the implementation of the 156-week Post-treatment Follow-up Period, it has been clarified that the clinical study report (CSR) for marketing authorization application (MAA) will be including treatment-emergent adverse events (TEAEs) reported for 9 weeks post last dose (as per the original version of the protocol). A final CSR will be issued at the End of the Trial.

Revised Sections: Section 9.9.2.1, Section 9.9.2.2, and Protocol Summary.

1.8. Adverse Events Section

1.8.1. AE recording after Treatment Discontinuation

Rationale:

CCI [redacted], it has been clarified that "*AEs that are suspected to be related to the IP as well as new malignancies or pre-malignancies regardless of causality (see Section 10.5.2) will be recorded by the Investigator until 156 weeks after the last dose of IP. Information will be collected as described in Table of Events, Table 3 and Section 6.5.*"

Revised Section: Section 10.1

1.8.2. Created Malignancy reporting subsection

Rationale:

CCI [redacted], a malignancy reporting subsection has been created into the Adverse Event Section. In this section a description of the malignancy monitoring is provided together with information on the reporting period.

Revised Section: Section 10.5.2

CCI [redacted]

1.9. Discontinuations

1.9.1. Treatment Discontinuation for any new malignancy

Rationale:

CCI [redacted], it has been clarified that subject should discontinue treatment for any new malignancy.

Revised Section: 11.1

1.10. Compound Background

1.10.1. Updated Potential Risks of Human Use section

Rationale:

Updated section based on updated clinical and nonclinical data.

Revised Section: Section 1.2.1.4

1.11. Other minor clarification and corrections

The amendment also includes several other minor clarifications and corrections:

- Clarification that re-screening is allowed and clarified timing of the re-screening procedures. Re-screening more than one time is allowed.
- The terminology Integrated Voice Response System (IVRS)/ Integrated Web Response System (IWRS) has been replaced with the term Interactive Response Technology (IRT) system. The IRT term encompasses all modalities when accessing the interactive system, thus for accuracy the term IRT has been updated throughout the protocol, instead of IVRS/IWRS
- Updated Potential Risk of Human Use section, as per the updated standard risk language for luspatercept (Section 1.2.1.4)
- Clarifications on local and central laboratory assessments provided for specific parameters in the Table of Events and Procedure sections.
- Clarifications for FCBP and males on the periods to be respected in regards to the pregnancy (Section 10.4.1 and Section 10.4.2)
- Dose Delay Section have been re-worded for clarity (Section 6.2.1 and Section 6.3.1)

- Clarification in Section 9.6.3.8 Duration of Transfusion Burden Response, to read "The duration of the ~~first~~ response…", since Section 9.6.3.9 Time to response covers the description on "first response", detailed analysis will be described in the SAP.
- Additional editorial revisions or minor clarifications