Official Protocol Title:	A Phase 2, Open-Label, Ascending Dose Study to Evaluate the Effects of ACE-536 in Patients with β-Thalassemia
NCT number:	NCT01749540
Document Date:	07-Nov-2014

CLINICAL STUDY PROTOCOL

A Phase 2, Open-Label, Ascending Dose Study to Evaluate the Effects of ACE-536 in Patients with β-Thalassemia

INVESTIGATIONAL PRODUCT:	**Luspatercept (ACE-536)**
PROTOCOL NUMBER:	**A536-04**
EudraCT NUMBER:	**2012-002499-15**
SPONSOR:	**Acceleron Pharma Inc.** **128 Sidney Street** **Cambridge, MA 02139 USA** **Tel:** PPD **Fax:** PPD
MEDICAL MONITOR:	PPD **Vice President, Medical Research**
ORIGINAL PROTOCOL DATE:	**06-Aug-2012**
AMENDMENT 01 DATE:	**07-Sep-2012**
AMENDMENT 02 DATE:	**09-Apr-2013**
AMENDMENT 03 DATE:	**27-Nov-2013**
AMENDMENT 04 DATE:	**07-Nov-2014**

ACCELERON PHARMA SIGNATURE PAGE

Signature: PPD

Date: 21 Nov 2014
DD/MMM/YYYY

Name (print):

By my signature, I indicate I have reviewed this protocol and find its content to be acceptable.

COORDINATING PRINCIPAL INVESTIGATOR SIGNATURE PAGE

Signature: 

Date: 9 DEC 2014
DD/MMM/YYYY

Name (print): Prof. Antonio Piga

Institution Name and Address:

University of Torino

Department of Clinical and Biological Sciences

Regione Gonzole 10 – 10043 Orbassano

Turin, Italy

By my signature, I agree the protocol has been written to comply with ICH Good Clinical Practices guidelines and agree to offer guidance throughout the study as needed.

I agree to conduct the study as outlined in the protocol. The study will be conducted in accordance to current United States Food and Drug Administration (FDA) regulations, International Conference of Harmonization (ICH) Guidelines, Good Clinical Practices (GCP), the Declaration of Helsinki, and local ethical and legal requirements.

SITE PRINCIPAL INVESTIGATOR SIGNATURE PAGE

Signature: _____ **Date:** _____
DD/MMM/YYYY

Name (print): _____

Institution Name and Address:

_____

_____

_____

By my signature I have read the protocol and agree to personally supervise and conduct the study as outlined in the protocol. The study will be conducted in accordance to current United States Food and Drug Administration (FDA) regulations, International Conference of Harmonization (ICH) Guidelines, Good Clinical Practices (GCP), the Declaration of Helsinki, and local ethical and legal requirements.

PROCEDURES IN CASE OF EMERGENCY

Table 1: Emergency Contact Information

Role in Study	Name	Contact Information
Acceleron Medical Monitor	PPD	Acceleron Pharma Inc. 128 Sidney Street Cambridge, MA 02139 USA PPD
Chiltern Medical Advisor	PPD	Chiltern 171 Bath Road Slough, Berkshire, SL1 4AA, UK PPD
Pharmacovigilance	Chiltern	PPD

1. PROTOCOL SYNOPSIS

Name of Sponsor/Company:	Acceleron Pharma Inc. 128 Sidney Street Cambridge, MA 02139 USA
Name of Investigational Product: Luspatercept (ACE-536)	
Name of Active Ingredient: ACE-536 is a recombinant fusion protein consisting of a modified form of the extracellular domain (ECD) of the human activin receptor IIB (ActRIIB) linked to the human IgG1 Fc domain.	
Title of Study: A phase 2, open-label, ascending dose study to evaluate the effects of ACE-536 in patients with β-thalassemia	
Study Centers: up to 15	
Phase of Development: 2	

Objectives:

- **Primary:**
  - To evaluate the proportion of β-thalassemia patients who have an erythroid response, defined as: 1) a hemoglobin increase of ≥ 1.5 g/dL from baseline for ≥ 14 days (in the absence of red blood cell [RBC] transfusions) in non-transfusion dependent patients, or 2) ≥ 20% reduction in RBC transfusion burden compared to pretreatment in transfusion dependent patients
- **Secondary:**
  - To evaluate safety and tolerability of ACE-536
  - To evaluate the proportion of transfusion dependent patients who have ≥ 50% reduction of RBC transfusion burden compared to pretreatment
  - To evaluate the time to erythroid response and duration of erythroid response
  - To evaluate the change in hemoglobin level in non-transfusion dependent patients
  - To evaluate the change in pre-transfusion hemoglobin levels in transfusion dependent patients
  - To evaluate changes in biomarkers of erythropoiesis, hemolysis, iron metabolism and bone metabolism
  - To examine the pharmacokinetic (PK) profile of ACE-536 in patients with β-thalassemia

- **Exploratory:**
    - To evaluate biomarkers related to the TGF-β superfamily
    - To evaluate self-reported quality of life using tools including but not limited to the FACT-An and SF-36 questionnaires (expansion cohort only)
    - To evaluate change in spleen size by MRI
    - To evaluate change in bone mineral density (BMD) by DXA
    - To evaluate change in extramedullary hematopoiesis (EMH) mass size by MRI
    - To evaluate change in leg ulcer size
    - To evaluate change in the 6-minute walk test (6MWT) distance in NTD patients

Methodology:

This is a phase 2, open-label, ascending dose study to evaluate the effects of ACE-536 in patients with β-thalassemia. Patients who meet the study eligibility criteria will be enrolled within 28 days of screening. Patients in all cohorts will receive ACE-536, administered subcutaneously (SC), every 3 weeks for up to 5 cycles. ACE-536 cannot be administered after study day 85 (± 2 days). Dose delay(s) and dose reduction(s) may be required for individual patients as outlined in the Patient Dose Modification Rules (Section 10.8.1).

Each dose escalation cohort will consist of up to 6 patients. The dose level of ACE-536 for the first cohort will be 0.2 mg/kg and the dose level(s) for subsequent cohort(s) will follow a modified Fibonacci dose escalation scheme (ie, maximum dose of 0.4 mg/kg for cohort 2, 0.6 mg/kg for cohort 3, 0.8 mg/kg for cohort 4, 1.0 mg/kg for cohort 5, etc.). Once a minimum of 3 patients in a cohort have completed Study Day 29, a Safety Review Team (SRT) will review preliminary safety and hematologic response data and make recommendations to the Sponsor regarding whether or not to enroll an additional cohort at a higher or lower dose, complete enrollment of 6 patients at the current dose level, and/or proceed to the expansion cohort.

The expansion cohort (n=30) will be treated with ACE-536 at a starting dose level not to exceed the maximum dose level for the previous cohorts. The expansion cohort will include a minimum of 12 patients who are non-transfusion dependent and a minimum of 12 patients who are transfusion dependent (including a minimum of 6 thalassemia major patients with onset of regular transfusions before age 4 years, and a minimum of 6 patients with onset of regular transfusions after age 4 years). For subsequent cycles in the expansion cohort, a patient's dose level may be titrated from the previous dose administered for that patient. Patients in the expansion cohort only may have their dose level titrated up or down as required per protocol to meet dose titration and modification rules (see Section 10.8). The maximum dose level given to a patient will not exceed the maximum dose level evaluated in the dose escalation cohorts. Patients will be treated with up to 5 doses of ACE-536.

Each TD patient in the expansion cohort will have a defined "pre-transfusion hemoglobin threshold" which will be calculated based on transfusion history and will be used for determining when to transfuse during the study. The baseline pre-transfusion hemoglobin threshold will be the mean of all documented pre-transfusion hemoglobin values during the 12 weeks prior to C1D1. During treatment, if the pre-transfusion hemoglobin value is increased by ≥ 1 g/dL compared to

the baseline pre-transfusion hemoglobin threshold for that patient, 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. Patients may be transfused at the investigator's discretion for symptoms related to anemia or other requirements (e.g., infection).

Cohort[a]	ACE-536 Dose Level[b] (mg/kg)	Number of Patients
1	0.2	3-6
2	0.4	3-6
3	0.6	3-6
4	0.8	3-6
5	1.0	3-6
6	1.25	3-6
7	1.5	3-6
Expansion	TBD	30[c]
Planned Total (All Cohorts):		**Approximately 51-72**

[a] Cohort escalation is based on SRT review and recommendation to enroll additional cohorts and/or the expansion cohort.

[b] The ACE-536 Dose Level for cohort 1 is 0.2 mg/kg. The dose level indicated for all subsequent cohorts is the maximum dose level that can be recommended by the SRT for escalation per the modified Fibonacci dose escalation scheme.

[c] The expansion cohort will consist of 30 patients, including a minimum of 12 patients who are non-transfusion dependent and a minimum of 12 patients who are transfusion dependent (including a minimum of 6 thalassemia major patients with onset of regular transfusions before age 4 years, and a minimum of 6 patients with onset of regular transfusions after age 4 years).

Maximum Number of Patients (Planned): 51-72 patients

Duration of Treatment: The total duration of participation for a patient is approximately 24 weeks (4 week screening period, 12 week treatment period, and 8 week follow up period). If a patient has a positive anti-drug antibody (ADA) result at the last visit, the patient may be asked to return for additional ADA testing every three months, until a negative result is obtained, or the result is considered to be stabilized.

Diagnosis and Main Criteria for Eligibility

Inclusion Criteria:

1. Men or women ≥ 18 years of age.
2. For the dose escalation phase of the study: documented diagnosis of β-thalassemia intermedia (transfusion dependent patients must not have begun regular transfusions at age < 4.0 years). For the expansion cohort: documented diagnosis of β-thalassemia (including β-thalassemia major or β-thalassemia intermedia).
3. Prior splenectomy or spleen size < 18 cm in the longest diameter by abdominal ultrasound (dose escalation cohorts only).

4. Anemia, defined as:
    a. Mean hemoglobin concentration < 10.0 g/dL of 2 measurements (one performed within one day prior to Cycle 1 Day 1 and the other performed during the screening period [Day -28 to Day -1]) in non-transfusion dependent patients, defined as having received < 4 units of RBCs within 8 weeks prior to Cycle 1 Day 1, OR
    b. Transfusion dependent, defined as requiring ≥ 4 units of RBCs every 8 weeks (confirmed over 6 months prior to Cycle 1 Day 1).
5. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) < 3 x upper limit of normal (ULN).
6. Serum creatinine ≤ 1.5 x ULN.
7. Females of child bearing potential (defined as sexually mature women who have not undergone hysterectomy or bilateral oophorectomy, or are not naturally postmenopausal ≥ 24 consecutive months) must have negative urine or blood pregnancy test prior to enrollment and use adequate birth control methods (abstinence, oral contraceptives, barrier method with spermicide, or surgical sterilization) during study participation and for 12 weeks following the last dose of ACE-536. Males must agree to use a latex condom during any sexual contact with females of child-bearing potential during study participation and for 12 weeks following the last dose of ACE-536, even if he has undergone a successful vasectomy. Patients must be counseled concerning measures to be used to prevent pregnancy and potential toxicities prior to the first dose of ACE-536.
8. Patients are able to adhere to the study visit schedule, understand and comply with all protocol requirements.
9. Understand and able to provide written informed consent.

Exclusion Criteria:

1. Any clinically significant pulmonary (including pulmonary hypertension), cardiovascular, endocrine, neurologic, hepatic, gastrointestinal, infectious, immunological (including clinically significant allo- or auto-immunization) or genitourinary disease considered by the investigator as not adequately controlled prior to Cycle 1 Day 1.
2. Folate deficiency.
3. Symptomatic splenomegaly.
4. Known positive for human immunodeficiency virus (HIV), active infectious hepatitis B (HBV), or active infectious hepatitis C (HCV).
5. Known history of thromboembolic events ≥ grade 3 according to the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) v.4.0 (current active minor version).
6. Ejection fraction < 50% by echocardiogram, MUGA or cardiac MRI.
7. Uncontrolled hypertension defined as systolic blood pressure (BP) ≥ 150 mm Hg or

diastolic BP ≥ 95 mm Hg.

8. Heart failure class 3 or higher (New York Heart Association, NYHA, Appendix 1).
9. QTc > 450 msec on screening ECG.
10. Platelet count < 100 x10^9/L or > 1,000 x10^9/L.
11. Proteinuria ≥ Grade 2.
12. Any active infection requiring parenteral antibiotic therapy within 28 days prior to Cycle 1 Day 1 or oral antibiotics within 14 days of Cycle 1 Day 1.
13. Treatment with another investigational drug or device, or approved therapy for investigational use ≤ 28 days prior to Cycle 1 Day 1, or if the half-life of the previous investigational product is known, within 5 times the half-life prior to Cycle 1 Day 1, whichever is longer.
14. Transfusion event within 7 days prior to Cycle 1 Day 1.
15. Patients receiving or planning to receive hydroxyurea treatment. Patients must not have had hydroxyurea within 90 days of Cycle 1 Day 1.
16. Splenectomy within 56 days prior to Cycle 1 Day 1.
17. Major surgery (except splenectomy) within 28 days prior to Cycle 1 Day 1. Patients must have completely recovered from any previous surgery prior to Cycle 1 Day 1.
18. Iron chelation therapy if initiated within 56 days prior to Cycle 1 Day 1.
19. Cytotoxic agents, systemic corticosteroids, immunosuppressants, or anticoagulant therapy such as warfarin or heparin within 28 days prior to Cycle 1 Day 1 (prophylactic aspirin up to 100 mg/day is permitted).
20. Pregnant or lactating females.
21. History of severe allergic or anaphylactic reactions or hypersensitivity to recombinant proteins or excipients in the investigational drug.
22. Prior treatment with sotatercept (ACE-011) or ACE-536.

Investigational Product and Mode of Administration:

ACE-536 drug product is provided as a sterile, liquid formulation at a nominal concentration of 50 mg/mL in Tris-buffered saline, pH 7.2 ± 0.5, contained in type I, 13-mm borosilicate glass vials (stoppered and crimp-sealed). Each single-use vial contains 0.5 mL (25 mg) of ACE-536 solution for injection.

ACE-536 will be administered by SC injection on Day 1 of each cycle.

Safety Evaluation and Dose Escalation:

Safety will be evaluated by the Safety Review Team (SRT), which is comprised at minimum of the coordinating investigator, medical monitor, and an independent hematologist. The role of the SRT will be described in greater detail in the SRT Charter. The SRT will review safety data when a minimum of 3 patients in a cohort have completed Study Day 29, including dose-limiting

toxicities (DLTs), adverse events (AEs), serious adverse events (SAEs), laboratory results (including hematology and chemistry), vital signs, and erythroid response data. The SRT may make one or more of the following recommendations to the sponsor:

- Enroll 3-6 patients in an additional cohort at a higher or lower dose level, not to exceed modified Fibonacci dose escalation scheme (see Section 9.1); enrollment in current cohort may be closed after a minimum of 3 patients.
- Proceed to the expansion cohort, with a recommended starting dose level for TD and NTD patients, not to exceed the maximum dose level evaluated in the dose escalation cohorts.
- Postpone a decision pending collection of additional data from the current cohort.
- Recommend that no further cohorts should be enrolled in the study.

A DLT, using the current active minor version of the National Cancer Institute Common Toxicity Criteria for Adverse Events, version 4.0 (NCI-CTCAE v4.0), is defined as any of the following toxicities at any dose level occurring within 28 days of the first administered dose:

- Treatment related serious adverse event (SAE) of ≥ Grade 3
- Treatment related non-hematologic adverse event (AE) of ≥ Grade 3
- Treatment related hematologic AE of ≥ Grade 4

Safety review team recommendations to escalate or reduce the dose level of ACE-536 in a subsequent cohort, or complete enrollment of 6 patients in the current cohort, will be based in part upon the following criteria:

- If a DLT occurs in ≤ 1 patient in a cohort within 28 days following the initial dose of ACE-536, dose escalation may occur;
- If a DLT occurs in ≥ 2 patients in a cohort within 28 days of the initial dose, the dose level for the next cohort (if any) should not be escalated; a lower dose may be recommended.
- If a hemoglobin increase of ≥ 2.0 g/dL (confirmed within 48 to 72 hours and not attributable to RBC transfusion) occurs in ≥ 2 patients in a cohort within 28 days of the initial dose, the dose level for the next cohort (if any) should not be escalated; a lower dose may be recommended.

Criteria for Evaluation

- **Safety:** All patients will be assessed for safety by monitoring adverse events, clinical laboratory tests, vital signs, ECG, and physical examination.
- **Efficacy:** Patients will be assessed for erythroid response for up to 20 weeks following initiation of treatment. Erythroid response endpoints will be determined by monitoring hematological laboratory values and RBC transfusions.

  Secondary efficacy endpoints will be assessed by examining other hematology, erythropoiesis, iron metabolism, and bone metabolism parameters, as well as MRI scan for liver iron content (LIC).

  - Erythropoiesis parameters include serum erythropoietin levels, hemoglobin analysis (electrophoresis, globin chain RNA), reticulocytes, nucleated RBCs, and RBC morphology
  - Hemolysis parameters include haptoglobin, indirect bilirubin, and lactate dehydrogenase (LDH)
  - Iron metabolism parameters include serum iron, total iron binding capacity (TIBC), transferrin, soluble transferrin receptor, ferritin, non-transferrin bound iron (NTBI), hepcidin, and LIC by MRI
  - Bone metabolism parameters include bone specific alkaline phosphatase (BSAP) and C-telopeptide of type I collagen (CTX)
- Exploratory endpoints will include:
  - Biomarkers related to the TGF-β superfamily
  - Self-reported quality of life measures including but not limited to the FACT-An and SF-36 questionnaires (expansion cohort only)
  - Spleen size by MRI
  - Bone mineral density (BMD) by DXA
  - Extramedullary hematopoiesis (EMH) mass size by MRI
  - Leg ulcer size
  - 6-minute walk test (6MWT) distance in NTD patients

Statistical methods

Sample Size Calculation:

There is no formal sample size calculation for the dose escalation portion of the study. A sample size of 30 evaluable patients in the expansion cohort will provide approximately 87% power with 1-sided significance level of 0.05 to differentiate an erythroid response rate of 30% from a minimal erythroid response rate of 10%.

Analysis Populations:

For all analysis populations, patients will be analyzed according to assigned cohort.

The Intent-to-Treat (ITT) Population: All patients enrolled in the study.

Efficacy Evaluable (EE) Population: All patients administered at least one dose of ACE-536, and 1) at least 2 hemoglobin values ≥ 14 days apart post-treatment, not influenced by transfusion, in non-transfusion dependent patients; OR, 2) at least 12 weeks of transfusion frequency data prior to Cycle 1 Day 1 and following Cycle 1 Day 1 in transfusion dependent patients.

Safety Population: All patients who received at least 1 dose of ACE-536.

Pharmacokinetics Population: All patients who have received at least 1 dose of ACE-536 and have sufficient pharmacokinetic (PK) samples collected and assayed for PK analysis.

Efficacy/Pharmacodynamic Effects Analysis:

The **primary efficacy endpoint** is defined as the proportion of β-thalassemia patients who have an erythroid response, defined as 1) a hemoglobin increase of ≥ 1.5 g/dL from baseline for ≥ 14 days (in the absence of RBC transfusions) in non-transfusion dependent patients or 2) ≥ 20% reduction in RBC transfusion burden compared to pretreatment in transfusion dependent patients.

Transfusion burden will be defined as the ratio of RBC transfusion units (or mLs) transfused during an interval to the duration (days) of that interval, where the interval may be during the pretreatment period or the treatment plus follow-up period. The interval during the pretreatment period will be defined as the 12 weeks prior to Cycle 1 Day 1. An interval during the treatment plus follow-up period will be defined as any 12-week interval after Cycle 1 Day 1.

The erythroid response will be summarized using both a point estimate and its exact 95% confidence interval based on binomial distribution. The primary efficacy analysis will be performed using the EE population.

No direct comparison testing with concurrent or historical controls will be performed.

The **secondary efficacy/pharmacodynamic analysis** will be performed using the ITT and EE populations and will include:

- Erythroid response, as defined above for primary endpoint, performed using the ITT population.
- For non-transfusion dependent patients, the proportion of patients with an increase of ≥ 1.0 g/dL from pretreatment hemoglobin maintained ≥ 8 weeks.
- For non-transfusion dependent patients, the proportion of patients with an increase of ≥ 1.5 g/dL from pretreatment hemoglobin maintained ≥ 8 weeks.
- For transfusion dependent patients, the proportion of patients who have ≥ 50% reduction in RBC transfusion burden compared to pretreatment using 12-week intervals.
- For transfusion dependent patients, the proportion of patients who have ≥ 50% reduction in RBC transfusion burden compared to pretreatment using 8-week intervals.
- For transfusion dependent patients, the proportion of patients who have no RBC transfusions ≥ 8 weeks.
- Time to erythroid response and duration of erythroid response
- Change from pretreatment in transfusion burden in transfusion dependent patients

- Change in hemoglobin level in non-transfusion dependent patients
- Change in pre-transfusion hemoglobin levels in transfusion dependent patients
- Change from baseline in erythropoiesis parameters including serum erythropoietin levels, hemoglobin analysis (electrophoresis, globin chain RNA), reticulocytes, and nucleated RBCs
- Change from baseline in hemolysis parameters include haptoglobin, indirect bilirubin, and lactate dehydrogenase (LDH)
- Change from baseline in iron metabolism parameters include serum iron, total iron binding capacity (TIBC), transferrin, calculated transferrin saturation, soluble transferrin receptor, ferritin, non-transferrin bound iron (NTBI), hepcidin, and LIC by MRI
- Change from baseline in bone metabolism parameters include bone specific alkaline phosphatase (BSAP) and C-telopeptide of type I collagen (CTX)

The **exploratory endpoints** will include evaluation of biomarkers related to the TGF-β superfamily, quality of life, change in spleen size (in subset of patients with no prior splenectomy), change in BMD, change in EMH mass size, change in leg ulcer size, and change in the 6MWT distance in NTD patients.

All binary endpoints will be summarized using both a point estimate and its exact confidence interval based on the binomial distribution. The time-to-event type secondary endpoints will be analyzed using Kaplan-Meier method to estimate the survival curve and median time to event and 95% confidence interval.

Safety analysis: To assess clinical safety, adverse events, vital sign measurements, and clinical laboratory information will be summarized. Adverse Events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Changes from baseline for clinical laboratory values and vital signs will be summarized over time. Descriptive statistics and shift tables will be generated as appropriate.

Pharmacokinetics analysis: Non-compartmental PK parameters for ACE-536, such as maximum plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}), and area under the concentration/time curve (AUC), will be estimated. Dose proportionality may be assessed using the exposure data (e.g., C_{max}, AUC) after the first dose if sufficient dose levels are studied. Descriptive statistics will be provided for serum concentrations and PK parameters. The relationship between ACE-536 exposure and response (i.e., safety, efficacy, and biomarkers) may be explored, if appropriate.

Anti-drug antibody analysis: The results of anti-drug and neutralizing antibody testing for ACE-536 and human ActRIIB protein versus time will be presented. Exploratory analysis will be performed on the potential effect of anti-drug antibodies on ACE-536 PK and drug exposure if anti-drug antibody tests are deemed positive.

2. SCHEDULE OF EVENTS

	Screen	Treatment Period																Follow up period	
		Cycle 1				Cycle 2			Cycle 3			Cycle 4			Cycle 5			EOT[12]	EOS[13]
		C1D1[2]	C1D8	C1D11	C1D15	C2D1[2,15]	C2D8	C2D15	C3D1[2,15]	C3D8	C3D15	C4D1[2,15]	C4D8	C4D15	C5D1[2,15]	C5D8	C5D15		
	Day -28 to -1	Day 1	Day 8 (± 1d)	Day 11 (± 1d)	Day 15 (± 1d)	Day[14] 22 (± 2d)	Day 29 (± 2d)	Day 36 (± 2d)	Day[14] 43 (± 2d)	Day 50 (± 2d)	Day 57 (± 2d)	Day[14] 64 (± 2d)	Day 71 (± 2d)	Day 78 (± 2d)	Day[14] 85 (± 2d)	Day 92 (± 2d)	Day 99 (± 2d)	Day 113 (± 7d)	Day 141 (± 7d)
Informed consent	X																		
Inclusion/Exclusion	X	X																	
Medical history	X																		
QoL Questionnaires[19]	X														X			X[20]	X
Physical examination	X	X[23]				X			X			X			X			X[23]	X[23]
Vital signs[1]	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	
ECG (12 lead)	X					X												X	
MRI (Liver Iron Content)[17]	X[3]																	X	
MRI for EMH masses and spleen[24]	X[3]																	X	
DXA for BMD of total body, lumbar spine, and total hip[24]	X[3]																	X	
Leg Ulcer Assessment[25]	X	X				X			X			X			X			X	X
Abdominal Ultrasound[21]	X[3]																	X	
ECHO, MUGA or cardiac MRI	X[3]																		
6MWT[24]	X																	X	
Serum iron studies[4]	X	X	X		X	X			X			X			X			X	X
Serum folate and B_{12}	X					X			X			X			X			X	X
Erythropoietin levels	X	X				X						X						X	X
Hematology[5]	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Peripheral blood smear	X	X				X			X			X			X			X	
Serum chemistry[6]	X	X			X	X			X			X			X			X	X
Urinalysis and Urine Chemistry[7]	X	X				X						X						X	X
Anti-drug antibody[16]		X										X						X	X[16]
PK collection		X	X	X	X	X	X					X	X	X	X	X	X	X	X
PD Biomarkers[8]		X				X			X			X			X			X	X
Hemoglobin electrophoresis		X										X						X	
Globin mRNA sample		X										X						X	
Bone Biomarkers[9]		X										X						X	
Pregnancy test/menstrual history[10]	X					X			X			X			X			X	X[22]
Evaluate transfusion frequency/volume[11]	X	X				X			X			X			X			X	X
Concomitant medications and AEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer ACE-536[18]		X				X[14]			X[14]			X[14]			X[14]				

[1] **Vital signs:** Weight, heart rate, systolic and diastolic blood pressure, respiration rate, and temperature (measured in degrees Celsius). Height is measured only at Screening. If at any visit the systolic blood pressure is ≥ 150 mmHg, the diastolic blood pressure is ≥ 95 mmHg, and/or the absolute increase in either measure from baseline is ≥ 20 mmHg, perform one repeat of the blood pressure assessment after a minimum of 15 minutes.

[2] **Study procedures** must be done prior to administration of study drug.

[3] Screening MRI for Liver Iron Content, MRI of the chest and abdomen for EMH masses and spleen (expansion cohort only), BMD by DXA (expansion cohort only), abdominal ultrasound of the spleen (if no prior splenectomy; dose escalation cohorts only) and ECHO, MUGA or cardiac MRI can be performed up to 56 days prior to C1D1. If performed as part of standard of care, it does not need to be repeated.

[4] **Iron Studies:** Serum iron, TIBC, transferrin, soluble transferrin receptor, ferritin, and NTBI.

[5] **Hematology:** RBC, WBC with differential, hemoglobin, hematocrit, haptoglobin (optional), reticulocyte count, platelet count, MCV, MCH, MCHC, RDW, and nRBCs. On dosing days, hematology values are to be drawn and resulted (up to 1 day) prior to C1D1 (see Section 10.8.1 Patient Dose Modification Rules). Historical hemoglobin values will be collected for 12 weeks prior to C1D1. Historical transfusion history will be collected for 12 months prior to Cycle 1 Day 1. Baseline hemoglobin will be the average of two measurements; one measure performed within one day prior to Cycle 1 Day 1 and the other performed during the screening period (Day -28 to Day -1). Note: For any RBC transfusions received during the study, collect hemoglobin value just prior to transfusion.

[6] **Chemistry**: Sodium, potassium, chloride, carbon dioxide/bicarbonate (optional), AST, ALT, lactate dehydrogenase (LDH), total bilirubin, indirect bilirubin, alkaline phosphatase, blood urea nitrogen (BUN)/urea, creatinine, GGT, calcium, phosphorus, glucose, amylase, lipase, total protein, albumin, and uric acid.

[7] **Urinalysis by dipstick analysis:** pH, specific gravity, protein, glucose, ketones, blood, leukocyte esterase, and nitrite, with microscopic examination if indicated. Microalbumin and creatinine will be performed by the central lab.

[8] **PD Biomarkers:** Hepcidin, GDF15 and others to be determined.

[9] **Bone Biomarkers:** BSAP and CTX.

[10] **Pregnancy** test (urine or serum) and menstrual history is required prior to C1D1 for female patients of child bearing potential only.

[11] Transfusion history will be collected for 12 months prior to C1D1.

[12] **End of Treatment (EOT):** Should be performed 28 days (± 7 days) after the last dose of ACE-536. Patients who discontinue treatment early should complete the end of treatment visit at the time of discontinuation and complete the Day 141 follow up visit 28 days (± 7 days) after the EOT visit.

[13] **End of Study (EOS):** Should be performed 28 days (± 7 days) after the Day 113/EOT visit (56 days after the last dose of ACE-536).

[14] **Day 85** ± 2 days is the last possible study day that ACE-536 may be administered, regardless of the cycle.

[15] If a **dose delay** is required per the dose modification rules the patient will not be dosed. The patient will return weekly to assess hematology results and adverse events until the patient is eligible to administer the next dose of ACE-536.

[16] If the patient has a positive ADA result at their last assessment, the patient may be asked to return approximately every three months for additional testing until a negative result is obtained or the result is considered stabilized.

[17] MRI for liver iron content will be performed at selected sites.

[18] For the first dose of ACE-536, dosing should occur after a minimum of 7 days post-transfusion; subsequent doses should not be given within 24 hours of transfusion or planned transfusion.

[19] Administration of quality of life questionnaires, including but not limited to FACT-An and SF-36 is required for patients in the expansion cohort only.

[20] QoL questionnaires including but not limited to FACT-An and SF-36 should be completed at the EOT visit only for expansion cohort patients who discontinue treatment early.

[21] Abdominal ultrasound of the spleen is required for patients with no prior splenectomy in the dose escalation cohorts only. Patients in these cohorts with clinical signs of a change in spleen size or abnormality should have an abdominal ultrasound as needed throughout study.

[22] Pregnancy test is not required at Day 141/EOS.

[23] Physical exam should include an optional evaluation of gonadal size in males at C1D1, EOT, and EOS visits only.

[24] MRI of the chest and abdomen for EMH masses and spleen, DXA for BMD and 6MWT for NTD patients will be performed in the expansion cohort patients only, at selected sites.

[25] Patients with leg ulcers should have regular assessment of the leg ulcer(s) throughout the study. Photographs of the leg ulcer(s) should be obtained to document any changes in leg ulcer(s) size.

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4. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
6MWT	Six minute walk test
ABPI	Association of the British Pharmaceutical Industry
ActRIIB	Activin receptor IIB
ADA	Anti-drug antibody
AE	Adverse Event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
BFU-E	Burst forming units - erythroid
BMD	Bone mineral density
BMP	Bone morphogenetic protein
BSAP	Bone specific alkaline phosphatase
BP	Blood pressure
BUN	Blood urea nitrogen
CXDY	Cycle X Day Y
CFU-E	Colony forming units - erythroid
CI	Confidence interval
C_{max}	Maximum concentration
CREAT	Creatinine
CRF	Case report form
CRO	Contract research organization
CTX	C-telopeptide of type I collagen
DLT	Dose-limiting toxicity
DXA	Dual-energy X-ray absorptiometry
ECD	Extracellular domain
ECHO	Echocardiogram
ECG	Electrocardiogram
EE	Efficacy Evaluable
EMH	Extramedullary hematopoiesis
EOS	End of Study

Abbreviation	Definition
EPO	Erythropoietin
EOT	End of Treatment
FACS	Fluorescence activated cell sorting
FACT-An	Functional Assessment of Cancer Therapy-Anemia Scale
FDA	United States Food and Drug Administration
GCP	Good clinical practices
GDF	Growth and differentiation factor
GGT	Gamma-glutamyl transpeptidase
HbA	Adult hemoglobin
HbF	Fetal hemoglobin
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
ICF	Informed consent form
ICH	International conference on harmonisation
IEC	Independent ethics committee
IgG1	Immunoglobulin G1
ITT	Intent-to-Treat
IB	Investigator's brochure
K_D	Dissociation constant
LIC	Liver iron content
LDH	Lactate dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MRI	Magnetic resonance imaging
MUGA	Multi gated acquisition scan
NCI-CTCAE	National Cancer Institute-Common terminology criteria for adverse events
NYHA	New York Heart Associate
nRBCs	Nucleated red blood cells
NTBI	Non-transferrin bound iron

Abbreviation	Definition
PD	Pharmacodynamic
PHI	Protected health information
PK	Pharmacokinetic
QoL	Quality of life
RBC	Red blood cell
RDW	Red blood cell distribution width
RNA	Ribonucleic acid
SAE	Serious adverse event
SAP	Statistical analysis plan
SC	Subcutaneous
SD	Standard deviation
SRT	Safety review team
SF-36	Short form (36) health survey
SUSAR	Suspected unexpected serious adverse reaction
$T_{1/2}$	Elimination half-life
TGF-β	Transforming growth factor beta
TIBC	Total iron binding capacity
T_{max}	Time to maximum concentration
ULN	Upper limit of normal
WBC	White blood cell

5. ETHICS

5.1. Institutional Review Board

The investigator will submit this protocol, any protocol modifications, and the patient Informed Consent Form (ICF) to be used in this study to the appropriate IEC for review and approval. A letter confirming IEC approval of the protocol and ICF as well as a statement that the IEC is organized and operates according to Good Clinical Practice (GCP) and the applicable laws and regulations, must be forwarded to the sponsor prior to the enrollment of patients into the study. A copy of the approved ICF will also be forwarded to the sponsor. Appropriate reports on the progress of the study will be made to the IEC and the sponsor by the principal investigator in accordance with applicable governmental regulations and in agreement with the policy established by the sponsor.

5.2. Ethical Conduct of the Study

The sponsor and the investigator must comply with all instructions, regulations, and agreements in this protocol and in the applicable International Conference on Harmonisation (ICH) and GCP guidelines, and must also conduct the study in accordance with local regulations.

5.3. Patient Information and Consent

Informed written consent is required from each patient prior to any testing under this protocol, including screening tests and evaluations. The ICF, as specified by the study center's IEC, must follow the Protection of Human Patients regulations listed in the Code of Federal Regulations, Title 21, Part 50. The principles of informed consent in the Declaration of Helsinki should be implemented in this clinical study and should comply with local and national regulations. The consent forms must be in a language fully comprehensible to the prospective subject. Information should be given in both oral and written form whenever possible and deemed appropriate by the IEC.

The background of the proposed study and the benefits and risks of the procedures and study must be explained to the patients. It is the responsibility of the investigator to obtain consent and to provide the patient with a copy of the signed and dated ICF. Confirmation of a patient's informed consent must also be documented in the patient's medical record prior to any testing under this protocol, including screening tests and evaluations.

All ICFs used in this study must be approved by the appropriate IEC and by the Sponsor or designee. The ICF must not be altered without the prior agreement of the relevant IEC and the Sponsor.

5.4. Patient Data Protection

Prior to any testing under this protocol, including screening tests and evaluations, patients must authorize the release and use of protected health information (PHI), as required by local law.

The patient will not be identified by name in the case report form (CRF) or in any study reports. These reports will be used for research purposes only. The Sponsor, its designee, and various

government health agencies may inspect the records of this study. Every effort will be made to keep the patient's personal medical data confidential.

6. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Acceleron Pharma is the sponsor for this trial. The sponsor will serve as the medical monitor for the study. The sponsor or designee also will manage the conduct of the trial and provide for clinical monitoring, data management, biostatistics, and report writing. Clinical monitors will monitor each study center on a periodic basis and verify source documentation for each patient. The sponsor's pharmacovigilance representative will be responsible for timely reporting of Serious Adverse Events (SAEs) to health authorities as required.

7. INTRODUCTION

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 IgG1 Fc domain. The ActRIIB receptor and its ligands are members of the 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.[1] Thus, observations from pharmacology studies of ACE-536 or its murine analog RAP-536 in animal models provide significant insight into the potential of ACE-536 to treat human disease.

Members of the TGF-β family are reported to play a role in red blood cell (RBC) development, although the specific ligands and receptors that influence the process of erythropoiesis are not completely understood. In nonclinical experiments, ACE-536 has been shown to bind with high affinity to some TGF-β ligands (eg, GDF8, GDF11, BMP6, and activin B), but substantially less, or not at all, to others (eg, BMP10 and activin A). The emerging body of evidence on ACE-536 suggests that its mechanism of action is distinct from that of erythropoietin (EPO), and involves acceleration of the later maturation phase of erythrocyte development.

The nonclinical pharmacology data using ACE-536/RAP-536 demonstrate that the molecule can produce a rapid and robust erythroid response in a variety of settings, and that this response is due to its influence on the differentiation of hematopoietic cell types in the later stages of erythropoiesis. In contrast, EPO is active in the early, proliferative stage of erythropoiesis. ACE-536 has shown activity in animal models of various conditions in which anemia contributes significantly to disease morbidity, including anemia associated with ineffective erythropoiesis. In a murine model of β-thalassemia, RAP 536 showed significant improvements in hematologic indices (red cell count, hemoglobin, hematocrit, red cell distribution width, and red cell morphology). Additionally, hemolysis was significantly reduced in RAP-536 treated mice, as measured by reduction in serum total bilirubin. Importantly, the mechanism of RAP-536 action in β-thalassemia mice was shown to be related to the accelerated maturation of erythroid precursor cell populations, specifically, increases in the orthochromatic erythroblasts and reticulocytes in the bone marrow and spleen, with concomitant reductions in the earlier-stage basophilic erythroblast population.

7.1. Overview of Target Indication

The target indication for ACE-536 is for the treatment of symptomatic anemia in patients with β-thalassemia. Anemia in β-thalassemia arises as a consequence of three main components, (i) ineffective erythropoiesis, (ii) red cell hemolysis, and (iii) abnormal red cell morphology. The most important of these is ineffective erythropoiesis, which is characterized by erythroid hyperplasia and substantially increased rates of apoptosis of erythrocyte precursors in the bone marrow, particularly the polychromatophilic and orthochromatic erythroblasts.[2] Apoptotic mechanisms are known to play a physiological role in the process of normal erythrocyte development; it is possible that these mechanisms are exacerbated in β-thalassemia.[3] Ineffective erythropoiesis can lead to erythroid expansion in the bone marrow which in turn can lead to bony deformities and osteoporosis, making patients with β-thalassemia more susceptible to fractures. The acceleration of red blood cell destruction can also result in splenomegaly.[4]

Onset of anemia in β-thalassemia generally occurs between 6 and 24 months of age, corresponding to the switch from the γ-chain of fetal hemoglobin (HbF) the β-chains of adult hemoglobin (HbA). The exact prevalence of β-thalassemia in the world population is unknown. In the Eastern Mediterranean it is estimated that there are 10,000 cases annually and of these cases approximately 9,000 are transfusion dependent.[5] According to Orpha.net (Report Series 2 May 2012) the estimated prevalence of β-thalassemia in Europe is 1-9/1,000,000, with a higher prevalence in Mediterranean regions.

General care for patients with β-thalassemia requires substantial supplemental pediatric care, including careful attention to nutrition and monitoring for signs of infection, red cell transfusions to prevent the damage of chronic anemia, and iron chelation to prevent the damage of iron overload due to ineffective erythropoiesis and regular blood transfusion. In older patients with signs of iron overload, supportive care is typically required to address endocrine insufficiencies, metabolic bone disease, and cardiac failure. Children with β-thalassemia who are periodically transfused to maintain a hemoglobin value of 9.5 to 14 g/dL grow and develop normally.[6] However, these patients require lifelong transfusion support, and concomitant iron chelation therapy to maintain a reasonable quality of life. Iron chelation therapy itself, can be associated with a number of side-effects, including neurosensory toxicities and retardation of bone. Iron-related cardiotoxicity is the most common cause of mortality in patients with β-thalassemia.

Advances in prenatal diagnosis have reduced incidence rate of β-thalassemia in developed countries, but have had little impact on the global incidence rate.[7] Treatments that have the potential to attenuate ineffective erythropoiesis and correct the anemia that characterize the disease could provide significant clinical benefit to patients by reducing the need for periodic blood transfusions and associated iron chelation therapy. ACE-536 has the potential to provide benefit in a variety of conditions in which ineffective erythropoiesis contributes significantly to anemia and overall disease morbidity.

7.2. Summary of Nonclinical Studies

A brief summary of key findings from pharmacology and toxicology studies is provided below. A comprehensive review of ACE-536, as well as details regarding the information summarized below, is provided in the Investigator's Brochure (IB). The most recent version of the ACE-536 IB should be reviewed prior to initiating the study.

7.2.1. Pharmacology Studies

In vitro and in vivo pharmacology studies have been conducted with ACE-536 or its murine orthologue, RAP-536. The use of RAP-536 allows longer term pharmacology studies in rodents without the confounding influence of immune reactivity.

A total of 26 potential ligands within the TGF-β superfamily were assessed for their in vitro binding specificity and affinity to ACE-536 by surface plasmon resonance (Biacore). Among the ligands examined, GDF8, GDF11, BMP6, and activin B bound to ACE-536 with the highest affinities at 37°C (KD values in the sub-nanomolar range). Further studies using a cell line reporter gene assay to assess signaling through the activin receptor demonstrate that signaling by GDF11 and GDF8 was inhibited by ACE-536.

During the course of early nonclinical pharmacology studies, an observation of a rapid, robust, and sustained increase in erythropoiesis in animals treated with ACE-536 was made. The effect of ACE-536 on bone marrow erythroid progenitor cells in mice was assessed by FACS analysis. Results demonstrate that the increase in RBC results as a consequence of a decrease of basophilic erythroblasts and a coordinate increase in subsequent, later stage polychromatic and orthochromatic erythroblasts as well as nucleated reticulocytes in both the bone marrow and spleen. Separate studies have confirmed that the increase in RBC occurs without affecting the populations of erythropoietin (EPO) responsive cells (BFU-E and CFU-E).

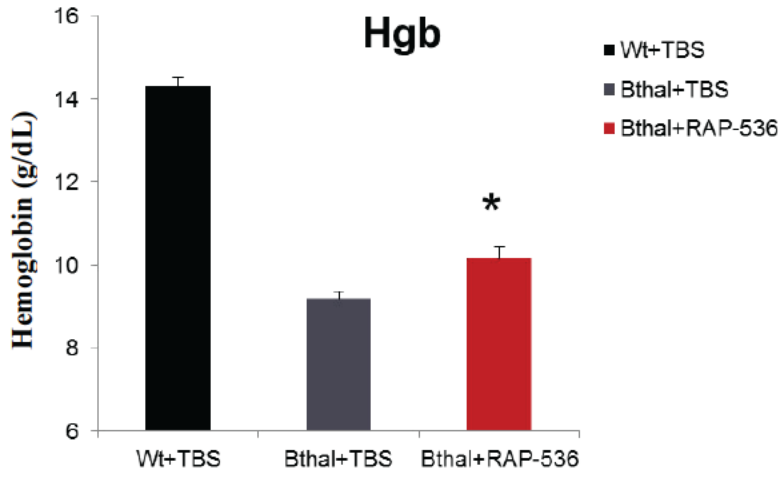
Fundamental to this application, a series of studies have been conducted in $Hbb^{major-/-}$ mice, a model of β-thalassemia in humans. These mice carry a deletion mutation in the β-globin gene, resulting in hematologic abnormalities very similar to those seen in human β-thalassemia intermedia, including severe anemia, ineffective erythropoiesis, splenomegaly and iron overload.[8] Results of studies in $Hbb^{major-/-}$ mice, described in more detail below, demonstrate that treatment with ACE-536 led to significant improvements in hematologic parameters, decreased hemolysis and reductions in spleen weight. Further, ACE-536 was shown to influence erythroid differentiation in the bone marrow and spleen of $Hbb^{major-/-}$ mice to increase the number of late-stage precursor cell populations.

RAP-536 was evaluated for its ability to improve ineffective erythropoiesis in thalassemic mice. $Hbb^{major-/-}$ mice, or their wild-type (Wt) counterparts, were treated with RAP-536 (1 mg/kg SC) or Tris-buffered saline (TBS) control, twice weekly for 8 weeks. Mice were 3 months of age at the initiation of the study and clearly exhibited features of β-thalassemia. At the end of the 8-week treatment period, assessment of hematologic parameters showed that RAP-536 treatment resulted in significant increases in red cell count, hemoglobin levels (Figure 1) and hematocrit, normalized red cell distribution width, and reduced red cell hemolysis, as measured by serum total bilirubin.

Importantly, the mechanism of RAP-536 action in $Hbb^{major-/-}$ mice was shown to be related to the accelerated maturation of erythroid precursor cell populations, specifically, increases in the orthochromatic erythroblasts and reticulocytes in the bone marrow and spleen, with concomitant reductions in the earlier-stage basophilic erythroblast population.

Concurrent with improvements in the hematology profile and in bone marrow/spleen cytology, Hbbmajor-/- mice treated with RAP-536 showed significant reductions in absolute spleen weight and as a percentage of body weight (data not shown). Finally, an unexpected finding from this study was an increase in bone mineral density, as measured by whole body DEXA scans, of Hbbmajor-/- mice treated with RAP-536. The mechanism for increased bone density is presently unclear, but could be related to normalization of bone metabolism associated with decreased anemia and concomitantly reduced marrow expansion cohort in RAP-536 treated animals, or possibly due to a direct effect of RAP-536 on bone turnover.

Figure 1: RAP-536 Improved Hemoglobin Levels in β Thalassemia Mice



*p ≤ 0.05 vs. Bthal + Tris-buffered Saline (TBS)

7.2.2. Toxicology Studies

Repeat-dose toxicology studies of 1- and 3-month treatment duration have been conducted with ACE 536 in Sprague-Dawley rats and cynomolgus monkeys to evaluate the toxicity of ACE-536. Recovery periods of up to 10 weeks were included as part of these studies. Findings from toxicology studies, described in more detail in the Investigator's Brochure, were different between rats and monkeys, though the expected pharmacologic effects of the drug were observed in both species.

ACE-536 exhibited favorable and predictable pharmacokinetic properties in both species. Serum concentrations of ACE-536 followed first-order absorption and first-order elimination kinetics after the first dose. The PK profiles of ACE-536 were similar among males and females, independent of dose level, and achieved peak or near peak mean serum concentrations at approximately 4 days in rats and 2 to 3 days in monkeys. The C_{max} and AUC were linear and approximately proportional to dose up to the highest dose levels evaluated. The serum terminal elimination half-life in rat is approximately 5 days and in monkey is 6 to 7 days, and clearance is independent of dose level and route of administration (SC vs IV).

Key findings identified in the 1- and 3-month rat toxicology studies included decreased heart, lung and prostate weight with no histopathology correlate, adrenal gland necrosis/congestion, and minimal liver necrosis. Stomach mineralization was only observed in the 1-month rat study.

Membranoproliferative glomerulonephritis was observed in rat and monkey studies, as well as increased BUN and creatinine. At the highest dose (30 mg/kg) in the monkey study, glomerulonephritis was accompanied by fibrosis and hemorrhage of the interstitium. The findings were suggestive of immune complex deposition, which could be associated with anti-drug antibodies; however, a direct effect of the drug cannot be excluded.

7.3. Summary of Clinical Experience

The safety, tolerability, pharmacokinetics, and pharmacodynamic effects of ACE-536 was evaluated in healthy postmenopausal women in a phase 1, randomized, double-blind, placebo controlled, multiple ascending dose study (Study A536-02). The primary objective of the study is to evaluate the safety and tolerability of ACE-536 in this population. Secondary objectives of the study are to examine the pharmacokinetic parameters and pharmacodynamic effects of ACE-536. Preliminary data from Study A536-02 as of 05 July 2012 are summarized below.

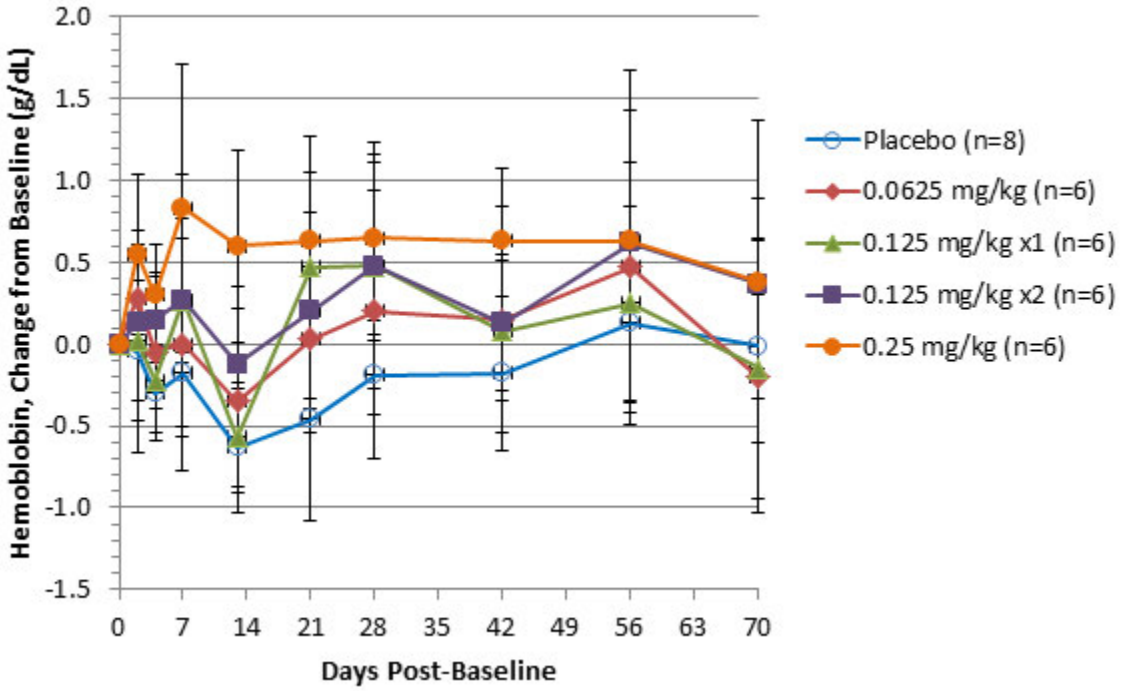
A total of 32 subjects were enrolled. The study consisted of 4 cohorts (0.0625 mg/kg x 2 doses, 0.125 mg/kg x 1 dose, 0.125 mg/kg x 2 doses, and 0.25 mg/kg x 2 doses). Subjects in each cohort were randomized and receive either ACE-536 or placebo (6 active and 2 placebo subjects per cohort). Doses were administered SC on Day 1 and Day 15. One subject in the third cohort and 5 subjects in the fourth cohort received only one dose of ACE-536.

The mean (SD) age for subjects in this study was 59.4 (5.8) years. Mean baseline hemoglobin level was 13.2 (0.6) g/dL. Preliminary analysis of PK data demonstrated that maximum ACE-536 concentration (C_{max}) and area under the curve ($AUC_{0\text{-}14d}$) after the first dose were generally dose-proportional from 0.0625 to 0.25 mg/kg. The mean time to maximum concentration (T_{max}) ranged from 7.7 to 11.7 days and the mean elimination half-life ($t_{½}$) ranged from 15.5 to 18.5 days. Pharmacokinetic results support SC dosing of ACE-536 every 3 weeks.

ACE-536 was generally safe and well-tolerated in this study of healthy postmenopausal female subjects. No serious or severe adverse events were reported. The majority of AEs were considered mild in severity. Adverse events that were considered probably or possibly related to study drug included injection site hemorrhage (3 subjects on active drug), injection site macule (2 subjects), and dry skin, hyperesthesia, muscle spasms, myalgia, generalized pruritis, and papular rash (1 subject each).

Consistent with results from the nonclinical pharmacology studies, ACE-536 administration resulted in a mean maximum increase in hemoglobin (at any timepoint during the study) of 1.3 g/dL in the 0.25 mg/kg group. The maximum hemoglobin increase from baseline at any timepoint for patients in the 0.25 mg/kg group ranged from 0.6 to 2.0 g/dL. The mean hemoglobin increase from baseline in that treatment group was at least 0.6 g/dL from Day 8 through Day 57, compared with a mean hemoglobin decrease from baseline in the placebo group of up to 0.6 g/dL through Day 43 (Figure 2).

Figure 2: Mean (±SD) Change from Baseline of Plasma Hemoglobin (g/dL) in Healthy Volunteers Treated with One or Two Doses of ACE-536



The preliminary results from this phase 1 study suggest a positive effect of 0.25 mg/kg ACE-536 on increasing hemoglobin in healthy subjects. Doses up to 0.25 mg/kg were generally safe and well-tolerated.

7.4. Potential Risks of Human Use

Increases in hematologic parameters (RBC, hemoglobin, hematocrit, reticulocytes) are expected pharmacologic effects of ACE-536 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 escalation/reduction rules will be utilized to minimize risks associated with increased RBC parameters.

Adverse events observed in the Phase 1 study in healthy volunteers that were considered probably or possibly related to study drug included injection site hemorrhage, injection site macule, dry skin, hyperesthesia, muscle spasms, myalgia, generalized pruritis, and papular rash.

Membranoproliferative glomerulonephritis was observed in both rats and monkeys, likely secondary to immune complex deposition. These findings were associated with increases in serum BUN and creatinine. The presence of immune complexes in renal tissue from monkeys treated with ACE-536 was demonstrated by immunohistochemistry.

The growth of heart and lungs was slightly reduced in rats exposed to ACE-536 as compared with control animals; these decreased heart and lung weights were reversible and there was no

corresponding histology finding. These findings were not replicated in the monkey toxicology studies; the potential for these adverse effects in humans is unknown.

Based on rat toxicology studies, treatment with ACE-536 may affect the adrenal gland. These findings were specific to the rat as they were not replicated in the monkey toxicology studies.

As with all biologics, there is the potential for anti-drug antibodies that can be associated with increased drug clearance and hypersensitivity reactions. Anti-drug antibody formation against ACE-536 as well as human ActRIIB protein will be monitored in the initial clinical studies.

No reproductive toxicity studies have been conducted with ACE-536 and therefore ACE-536 should not be administered to pregnant women. Male and female subjects of childbearing potential participating in studies of ACE-536 must be willing to abstain from sexual intercourse or use adequate contraception during the treatment and for 12 weeks following the last dose of ACE-536. The effects of ACE-536 on the developing fetus are unknown. It is not known if ACE-536 is secreted in milk; therefore, ACE-536 should not be administered to nursing mothers.

It is unknown if humans will experience any of the effects of ACE-536 that were noted in the rat and monkey toxicology studies. Safety effects will be monitored closely through adverse event reporting, clinical laboratory tests, vital signs, and physical examinations.

A comprehensive review of ACE-536, as well as details regarding the information summarized above, is provided in the Investigator's Brochure (IB). The ACE-536 IB should be reviewed prior to initiating the study.

8. TRIAL OBJECTIVES

8.1. Primary Objective

- To evaluate the proportion of β-thalassemia patients who have an erythroid response, defined as: 1) a hemoglobin increase of ≥ 1.5 g/dL from baseline for ≥ 14 days (in the absence of red blood cell [RBC] transfusions) in non-transfusion dependent patients, or 2) ≥ 20% reduction in RBC transfusion burden compared to pretreatment in transfusion dependent patients

8.2. Secondary Objectives

- To evaluate safety and tolerability of ACE-536
- To evaluate the proportion of transfusion dependent patients who have ≥ 50% reduction of RBC transfusion burden compared to pretreatment
- To evaluate the time to erythroid response and duration of erythroid response
- To evaluate the change in hemoglobin level in non-transfusion dependent patients
- To evaluate the change in pre-transfusion hemoglobin levels in transfusion dependent patients
- To evaluate changes in biomarkers of erythropoiesis, hemolysis, iron metabolism, and bone metabolism
- To examine the pharmacokinetic (PK) profile of ACE-536 in patients with β-thalassemia

8.3. Exploratory Objective

- To evaluate biomarkers related to the TGF-β superfamily
- To evaluate self-reported quality of life using tools including but not limited to the FACT-An and SF-36 questionnaires (expansion cohort only)
- To evaluate change in spleen size by MRI
- To evaluate change in bone mineral density (BMD) by DXA
- To evaluate change in extramedullary hematopoiesis (EMH) mass size by MRI
- To evaluate change in leg ulcer size
- To evaluate change in the 6-minute walk test (6MWT) distance in NTD patients

9. OVERALL STUDY DESIGN AND PLAN: DESCRIPTION

This is a phase 2, open-label, ascending dose study to evaluate the effects of ACE-536 in patients with β-thalassemia.

9.1. Study Design

Patients who meet the study eligibility criteria will be enrolled within 28 days of screening. Patients in all cohorts will receive ACE-536, administered subcutaneously (SC), every 3 weeks for up to 5 cycles. ACE-536 cannot be administered after study day 85 (± 2 days). Dose delay(s) and dose reduction(s) may be required for individual patients as outlined in the Patient Dose Modification Rules (Section 10.8.1).

Each dose escalation cohort will consist of up to 6 patients. The dose level of ACE-536 for the first cohort will be 0.2 mg/kg and the dose level(s) for subsequent cohort(s) will follow a modified Fibonacci dose escalation scheme (i.e., maximum dose of 0.4 mg/kg for cohort 2, 0.6 mg/kg for cohort 3, 0.8 mg/kg for cohort 4, 1.0 mg/kg for cohort 5, etc.). Once a minimum of 3 patients in a cohort have completed Study Day 29, a Safety Review Team (SRT) will review preliminary safety and hematologic response data and make recommendations to the Sponsor regarding whether or not to enroll an additional cohort at a higher or lower dose, complete enrollment of 6 patients at the current dose level, or proceed to the expansion cohort.

The expansion cohort (n=30) will be treated with ACE-536 at a starting dose level not to exceed the maximum dose level for the previous cohorts. The expansion cohort will include a minimum of 12 patients who are non-transfusion dependent and a minimum of 12 patients who are transfusion dependent (including a minimum of 6 thalassemia major patients with onset of regular transfusions before age 4 years, and a minimum of 6 patients with onset of regular transfusions onset after age 4 years). For subsequent cycles in the expansion cohort, a patient's dose level may be titrated from the previous dose administered for that patient. Patients in the expansion cohort only may have their dose level titrated up or down as required per protocol to meet dose titration and modification rules (see Section 10.8). The maximum dose level given to a patient will not exceed the maximum dose level evaluated in the dose escalation cohorts. Patients will be treated with up to 5 doses of ACE-536.

Each TD patient in the expansion cohort will have a defined "pre-transfusion hemoglobin threshold" which will be calculated based on transfusion history and will be used for determining when to transfuse during the study. The baseline pre-transfusion hemoglobin threshold will be the mean of all documented pre-transfusion hemoglobin values during the 12 weeks prior to C1D1. During treatment, if the pre-transfusion hemoglobin value is increased by ≥ 1 g/dL compared to the baseline pre-transfusion hemoglobin threshold for that patient, 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. Patients may be transfused at the investigator's discretion for symptoms related to anemia or other requirements (e.g., infection).

Cohort[a]	ACE-536 Dose Level[b] (mg/kg)	Number of Patients
1	0.2	3-6
2	0.4	3-6
3	0.6	3-6
4	0.8	3-6
5	1.0	3-6
6	1.25	3-6
7	1.5	3-6
Expansion	TBD	30[c]
Planned Total (All Cohorts):		**Approximately 51-72**

[a] Cohort escalation is based on SRT review and recommendation to enroll additional cohorts and/or the expansion cohort.
[b] The ACE-536 dose level for cohort 1 is 0.2 mg/kg. The dose level indicated in the table for all subsequent cohorts is the maximum dose level that can be recommended by the SRT for escalation per the modified Fibonacci dose escalation scheme.
[c] The expansion cohort will consist of 30 patients, including a minimum of 12 patients who are non-transfusion dependent and a minimum of 12 patients who are transfusion dependent (including a minimum of 6 thalassemia major patients with onset of regular transfusions before age 4 years, and a minimum of 6 patients with onset of regular transfusions after age 4 years).

9.2. Discussion of Study Design

The primary efficacy endpoint, evaluating sustained increase in hemoglobin during treatment with ACE-536, addresses the primary deficiency in β-thalassemia, namely profound anemia particularly marked in thalassemia major, but clearly present also in thalassemia intermedia and largely driving the clinical picture. A therapy capable of maintaining or increasing hemoglobin levels with chronic treatment can reverse the symptoms associated with anemia, reduce transfusion requirement, decrease iron overload, and decrease the need for iron chelation and the associated adverse effects of chelation treatment. ACE-536 treatment may prove effective in promoting erythroid maturation and reducing ineffective erythropoiesis. It may have additional salutary effects on iron metabolism and bone defects. It is unknown if ACE-536 will also affect the globin chain imbalance characteristic of the disease. Thus, the secondary endpoints were selected to evaluate the impact of ACE-536 on hematology, erythropoiesis, iron metabolism, and bone metabolism parameters.

The initial dose level of 0.2 mg/kg is less than the maximum dose administered in the phase 1 study A536-02 of 0.25 mg/kg, which was safe and well-tolerated, and produced a hemoglobin response in healthy volunteers. The safety measurements in this study are standard for studies with investigational medications. An open-label study, conducted in a limited number of patients, with protocol-specified definitions of dose-limiting toxicity, and with protocol-specified procedures for dose escalation and reduction is considered appropriate for a proof-of-concept study in this target patient population. Dose escalation in sequential cohorts is designed to minimize the risk of adverse events as well as excessive erythroid response and hypertension that may result from a robust response to high drug levels. Individualization of dose level in the expansion cohort, using dose titration rules based on hemoglobin response and dose reduction

rules based on safety information will maximize the proportion of patients having an erythroid response while maintaining safety.

9.3. Selection of Study Population

9.3.1. Inclusion Criteria

Eligible patients must meet **all** of the following criteria:

1. Men or women ≥ 18 years of age.
2. For the dose escalation phase of the study: documented diagnosis of β-thalassemia intermedia (transfusion dependent patients must not have begun regular transfusions at age < 4.0 years)
   For the expansion cohort: documented diagnosis of β-thalassemia (including β-thalassemia major or β-thalassemia intermedia).
3. Prior splenectomy or spleen size < 18 cm in the longest diameter by abdominal ultrasound (dose escalation cohorts only).
4. Anemia, defined as:
   a. Mean hemoglobin concentration < 10.0 g/dL of 2 measurements (one performed within one day prior to Cycle 1 Day 1 and the other performed during the screening period [Day -28 to Day -1]) in non-transfusion dependent patients, defined as having received < 4 units of RBCs within 8 weeks prior to Cycle 1 Day 1, OR
   b. Transfusion dependent, defined as requiring ≥ 4 units of RBCs every 8 weeks (confirmed over 6 months prior to Cycle 1 Day 1).
5. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) < 3 x upper limit of normal (ULN).
6. Serum creatinine ≤ 1.5 x ULN.
7. Females of child bearing potential (defined as sexually mature women who have not undergone hysterectomy or bilateral oophorectomy, or are not naturally postmenopausal ≥ 24 consecutive months) must have negative urine or blood pregnancy test prior to enrollment and use adequate birth control methods (abstinence, oral contraceptives, barrier method with spermicide, or surgical sterilization) during study participation and for 12 weeks following the last dose of ACE-536. Males must agree to use a latex condom during any sexual contact with females of child-bearing potential during study participation and for 12 weeks following the last dose of ACE-536, even if he has undergone a successful vasectomy. Patients must be counseled concerning measures to be used to prevent pregnancy and potential toxicities prior to the first dose of ACE-536.
8. Patients are able to adhere to the study visit schedule, understand and comply with all protocol requirements.
9. Understand and able to provide written informed consent.

9.3.2. Exclusion Criteria

Eligible patients must not meet **any** of the following criteria:

1. Any clinically significant pulmonary (including pulmonary hypertension), cardiovascular, endocrine, neurologic, hepatic, gastrointestinal, infectious, immunological (including clinically significant allo- or auto-immunization), or genitourinary disease considered by the investigator as not adequately controlled prior to Cycle 1 Day 1.
2. Folate deficiency.
3. Symptomatic splenomegaly.
4. Known positive for human immunodeficiency virus (HIV), active infectious hepatitis B (HBV), or active infectious hepatitis C (HCV).
5. Known history of thromboembolic events ≥ grade 3 according to the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) v.4.0 (current active minor version).
6. Ejection fraction < 50% by echocardiogram, MUGA or cardiac MRI.
7. Uncontrolled hypertension defined as systolic blood pressure (BP) ≥ 150 mm Hg or diastolic BP ≥ 95 mm Hg.
8. Heart failure class 3 or higher (New York Heart Association, NYHA, Appendix 1).
9. QTc > 450 msec on screening ECG.
10. Platelet count < 100 $x10^9$/L or > 1,000 $x10^9$/L.
11. Proteinuria ≥ Grade 2.
12. Any active infection requiring parenteral antibiotic therapy within 28 days prior to Cycle 1 Day 1 or oral antibiotics within 14 days of Cycle 1 Day 1.
13. Treatment with another investigational drug or device, or approved therapy for investigational use ≤ 28 days prior to Cycle 1 Day 1, or if the half-life of the previous investigational product is known, within 5 times the half-life prior to Cycle 1 Day 1, whichever is longer.
14. Transfusion event within 7 days prior to Cycle 1 Day 1.
15. Patients receiving or planning to receive hydroxyurea treatment. Patients must not have had hydroxyurea within 90 days of Cycle 1 Day 1.
16. Splenectomy within 56 days prior to Cycle 1 Day 1.
17. Major surgery (except splenectomy) within 28 days prior to Cycle 1 Day 1. Patients must have completely recovered from any previous surgery prior to Cycle 1 Day 1.
18. Iron chelation therapy initiated within 56 days prior to Cycle 1 Day 1.
19. Cytotoxic agents, systemic corticosteroids, immunosuppressants, or anticoagulant therapy such as warfarin or heparin within 28 days prior to Cycle 1 Day 1 (prophylactic aspirin up to 100 mg/day is permitted).

20. Pregnant or lactating females.
21. History of severe allergic or anaphylactic reactions or hypersensitivity to recombinant proteins or excipients in the investigational drug.
22. Prior treatment with sotatercept (ACE-011) or ACE-536.

9.4. Patient Treatment Discontinuation and Withdrawal Criteria

Patients will be informed that they have the right to withdraw from the study at any time for any reason without prejudice to their medical care.

A patient may be discontinued from treatment for any of the following reasons:

- Patient's request
- Patient's unwillingness or inability to comply with the protocol
- Pregnancy
- Use of prohibited medication (e.g., hydroxyurea)
- Medical reason or adverse event, at the discretion of the investigator and/or the medical monitor
- Hypersensitivity reaction to study drug
- At the discretion of the sponsor (e.g., termination of the study or a dose level)

A patient may be withdrawn from the study for any of the following reasons:

- Patient's request
- Patient's unwillingness or inability to comply with the protocol
- Death
- Loss to follow-up
- At the discretion of the sponsor (e.g., termination of the study)

The reasons for study withdrawal and treatment discontinuation must be recorded in the patient's CRF. The investigator must notify the sponsor, the medical monitor and the contract research organization (CRO) immediately when a patient has been discontinued/withdrawn due to an AE. Patients who discontinue treatment early should complete the end of treatment (EOT) follow-up visit at the time of discontinuation and then complete the end of study (EOS) follow-up visit approximately 28 days later.

The investigator must notify the sponsor and the CRO when a patient has been discontinued/withdrawn for reasons unrelated to the study or study drug (e.g., withdrawn consent, lost to follow up).

10. TREATMENT OF PATIENTS

10.1. Selection and Timing of Dose for Each Patient

Once a patient is enrolled, the appropriate dose of ACE-536 will be administered as a subcutaneous injection on Cycle 1 Day 1. Subsequent doses will be administered every 3 weeks on Day 1 of the cycle. Study day 85 (± 2 days) is the last possible study day that ACE-536 can be administered, regardless of the cycle. Dose reductions may be required for individual patients as outlined in the Patient Dose Modifications Rules (Section 10.8.1). If a dose delay is required, the patient will return weekly for assessment of hematology results and adverse events until the patient is eligible to receive the next dose of ACE-536. Patients will be asked to return to the clinic for two follow-up visits, occurring approximately 28 days and 56 days after their last dose of ACE-536. If a patient has a positive anti-drug antibody (ADA) result at the last visit, the patient may be asked to return for additional ADA testing every three months, until a negative result is obtained or the result is considered to be stabilized.

10.2. Concomitant Medications

During screening and throughout the study patients may take stable doses of medications for chronic conditions that are not specifically excluded by the protocol (see Section 9.3.1 and Section 9.3.2). If a patient requires treatment with any new medications that are specifically excluded by the eligibility criteria, the patient will be discontinued from the study and should complete the end of treatment visit and enter the follow-up period of the study. 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. Concomitant medications will be collected beginning at study screening and will include all medications taken within 28 days prior to Cycle 1 Day 1.

10.2.1. Other Treatments for β-Thalassemia

If treatment with hydroxyurea is required during the ACE-536 treatment period, the patient should be discontinued from treatment with study drug and complete the end of treatment visit and enter the follow-up period. Iron chelation therapy is allowed if initiated at least 56 days prior to C1D1.

10.2.2. 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 (e.g., hemodynamic or pulmonary compromise requiring treatment) or a comorbidity.

For any RBC transfusions received during the study, collect hemoglobin values just prior to transfusion.

Each TD patient in the expansion cohort will have a defined “pre-transfusion hemoglobin threshold” which will be calculated based on transfusion history and will be used for determining when to transfuse during the study. The baseline pre-transfusion hemoglobin threshold will be the mean of all documented pre-transfusion hemoglobin values during the 12 weeks prior to

C1D1. During treatment, if the pre-transfusion hemoglobin value is increased by ≥ 1 g/dL compared to the baseline pre-transfusion hemoglobin threshold for that patient, 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. Patients may be transfused at the investigator's discretion for symptoms related to anemia or other requirements (e.g., infection).

For the first dose of ACE-536, dosing should occur after a minimum of 7 days post-transfusion; subsequent doses should not be given within 24 hours of transfusion or planned transfusion.

10.3. Treatment Compliance

ACE-536 will be administered as a SC injection at the clinical site by the study staff and will be documented in the study record. Monitoring for patient compliance with the treatment regimen is therefore unnecessary.

10.4. Randomization

This is an open-label study that does not require randomization.

10.5. Treatments Administered

Patients will continue to receive the same dose level of study drug as they were assigned at study entry unless a dose modification is required (see Section 10.8).

10.6. Dose-Limiting Toxicity Definition

A Dose-Limiting Toxicity (DLT), using the current active minor version of the National Cancer Institute Common Toxicity Criteria for Adverse Events version 4.0 (NCI-CTCAE v4.0), is defined as any of the following toxicities, at any dose level occurring within 28 days of the first administered dose:

- Treatment related serious adverse event (SAE) of Grade ≥ 3
- Treatment related non-hematologic adverse event (AE) of Grade ≥ 3
- Treatment related hematologic AE of Grade ≥ 4.

10.7. Safety Evaluation and Dose Escalation

Safety will be evaluated by the Safety Review Team (SRT), which is comprised at minimum of the coordinating investigator, medical monitor, and an independent hematologist. The role of the SRT will be described in greater detail in the SRT Charter. The SRT will review safety data when a minimum of 3 patients in a cohort have completed Study Day 29, including dose-limiting toxicities (DLTs), adverse events (AEs), serious adverse events (SAEs), laboratory results (including hematology and chemistry), vital signs, and erythroid response data. The SRT may make one or more of the following recommendations to the sponsor:

- Enroll 3-6 patients in an additional cohort at a higher or lower dose level, not to exceed modified Fibonacci dose escalation scheme (see Section 9.1); enrollment in current cohort may be closed after a minimum of 3 patients.

- Proceed to the expansion cohort, with a recommended starting dose level for TD and NTD patients, not to exceed the maximum dose level evaluated in the dose escalation cohorts.
- Postpone a decision pending collection of additional data from the current cohort.
- Recommend that no further cohorts should be enrolled in the study.

SRT recommendations to escalate or reduce the dose level of ACE-536 in a subsequent cohort, or complete enrollment of 6 patients in the current cohort, will be based in part upon the following criteria:

- If a DLT occurs in ≤ 1 patient in a cohort within 28 days following the initial dose of ACE-536, dose escalation may occur;
- If a DLT occurs in ≥ 2 patients in a cohort within 28 days of the initial dose, the dose level for the next cohort (if any) should not be escalated; a lower dose may be recommended.
- If a hemoglobin increase of ≥ 2.0 g/dL (confirmed within 48 to 72 hours and not attributable to RBC transfusion) occurs in ≥ 2 patients in a cohort within 28 days of the initial dose, the dose level for the next cohort (if any) should not be escalated; a lower dose may be recommended.

10.8. Patient Dose Modification and Titration

Patients in the dose escalation cohorts have a pre-determined dose level based on cohort number (see Section 9.1). Dose level titration is not allowed for patients in the dose escalation cohorts, but dose delay or reduction may be required according to Patient Dose Modification Rules (Section 10.8.1). Examples of dose level reductions for patients in dose escalation cohorts are included in Table 2 below. Patients who require more than 2 dose reductions due to an AE should be discontinued from treatment and complete the end of treatment and end of study visits.

All patients in the expansion cohort will be initially treated with ACE-536 at an assigned starting dose level (to be determined) which has been deemed to be safe, well tolerated, and at least minimally effective by the SRT. Examples of possible expansion cohort starting dose levels and associated modifications (reductions and titrations) are in Table 2. Rules for individual patient titrations are described in Section 10.8.2 for NTD patients and Section 10.8.3 for TD patients.

Table 2: Starting Dose Levels, Dose Level Reductions (All Cohorts), and Dose Level Titrations (Expansion Cohort Only)

2nd Dose Reduction	1st Dose Reduction	Starting Dose Level	1st Dose Titration	2nd Dose Titration
0.2 mg/kg	0.4 mg/kg	**0.6 mg/kg**	0.8 mg/kg	1.0 mg/kg
0.4 mg/kg	0.6 mg/kg	**0.8 mg/kg**	1.0 mg/kg	1.25 mg/kg
0.6 mg/kg	0.8 mg/kg	**1.0 mg/kg**	1.25 mg/kg	1.5 mg/kg
0.8 mg/kg	1.0 mg/kg	**1.25 mg/kg**	1.5 mg/kg	N/A
1.0 mg/kg	1.25 mg/kg	**1.5 mg/kg**	N/A	N/A

- Dose titration to higher dose levels is only applicable to patients in the expansion cohort.
- Expansion cohort starting dose level for TD and NTD patients to be determined by the sponsor in conjunction with the SRT (range: 0.2-1.5 mg/kg).
- Dose titration will not exceed the maximum dose level tested in the dose escalation cohorts and determined to be safe and well tolerated by the SRT.
- Patients may increase and decrease dose level per protocol as needed, however patients who require more than 2 dose reductions due to an AE should be discontinued from treatment and complete the end of treatment and end of study visits.

10.8.1. Patient Dose Modification Rules

The following dose modification rules include both pharmacodynamic and safety parameters which may require a dose delay and possibly a dose reduction. These rules should be assessed prior to dosing. If a dose delay is required the patient should have weekly visits to assess hematology results and adverse events until the patient is eligible to receive the next dose of ACE-536.

Observation on Dosing Day	Action	ACE-536 Dose Modification[a]
Hemoglobin ≥ 13 g/dL	Hold dose and monitor patient weekly until hemoglobin < 12 g/dL	Resume dosing once hemoglobin < 12 g/dL.
Hemoglobin increase ≥ 2 g/dL from the previous dosing day (not attributable to RBC transfusion)	Continue dosing	Reduce dose by 1 dose level[b]
Non-hematologic adverse event ≥ Grade 3, or hematologic adverse event ≥ Grade 4, at least possibly related to study drug	Hold dose and monitor patient weekly until resolution of AE to ≤ Grade 1 or baseline	Resume dosing upon resolution of the AE to ≤ Grade 1 or baseline and reduce dose level by 1 dose level[b]

[a] Last possible treatment day is Study Day 85 (± 2 days).
[b] All subsequent doses will remain at the new dose level or can be reduced further if needed. Patients who require more than 2 dose reductions due to an AE should be discontinued from treatment and complete the end of treatment and end of study visits.

10.8.2. Individual Dose Titration Rules for NTD Patients in the Expansion Cohort

For the expansion cohort, the starting dose to be administered on Cycle 1 Day 1 for NTD patients will be determined by the sponsor in conjunction with the SRT (see Table 2). For subsequent cycles, dose level may be titrated individually for each patient. For all NTD patients the dose level will be assessed for titration at C3D1, C4D1, and C5D1. The dose level should be titrated individually for each patient not to exceed the maximum dose level evaluated as follows:

- If hemoglobin increase from baseline is < 1.5 g/dL throughout the previous 2 cycles at the same dose level, the dose level will be increased by 1 dose level (unless dose modification is required).
- If hemoglobin increase from baseline is ≥ 1.5 g/dL but not sustained for at least 2 consecutive measurements during the previous 2 cycles at the same dose level, the dose level will be increased by 1 dose level (unless dose modification is required).
- If hemoglobin increase from baseline is ≥ 1.5 g/dL for at least 2 consecutive measurements during the previous 2 cycles at the same dose level, the dose level will be unchanged (unless dose modification is required).

Baseline hemoglobin to assess titration will be the average of 2 or more measurements performed during the screening period. Hemoglobin measurements within 2 weeks following RBC transfusion will be excluded from the both the calculated mean baseline hemoglobin and on study values used for evaluating dose titration for a patient.

10.8.3. Individual Dose Titration Rules for TD Patients in the Expansion Cohort

For the expansion cohort, the starting dose to be administered on Cycle 1 Day 1 for TD patients will be determined by the sponsor in conjunction with the SRT (see Table 2Table 2). For subsequent cycles, the dose level may be titrated individually for each patient. For all TD patients the dose level will be assessed for titration at C3D1, C4D1, and C5D1. The dose level should be titrated individually for each patient not to exceed the maximum dose level evaluated as follows:

- If patient is transfused with ≥ 3 units during the previous 2 cycles at the same dose level, the dose level will be increased by 1 dose level (unless a dose modification is required)

A dose delay and/or dose reduction may be required for individual patients as outlined in the Patient Dose Modification Rules (Section 10.8.1). The SRT will meet periodically to monitor overall safety and erythroid response data for the expansion cohort.

10.8.4. Other Considerations for Dose Modification, Delay or Discontinuation

For individual patients judged by the investigator to be at an unacceptable risk, but who do not meet the protocol-defined conditions for a dose modification, the investigator should consult with the medical monitor to decide whether to continue dosing at the same dose level, reduce the dose level, or discontinue the patient's treatment with ACE-536.

11. STUDY PROCEDURES

Please refer to Section 2 for the schedule of procedures required for each visit.

11.1. Written Informed Consent

Patients will be required to sign an IEC approved ICF prior to any study related procedures, including screening evaluations.

11.2. Clinical Laboratory Tests

The following laboratory assessments will be performed at the clinical site's local laboratory according to the laboratory collection recommendations. The sponsor may request additional safety tests be performed based on ongoing data review during the study.

- Hematology: RBC, WBC with differential, hemoglobin, hematocrit, haptoglobin (optional), reticulocyte count, platelet count, MCV, MCH, MCHC, and RDW.
- Serum chemistry: Sodium, potassium, chloride, carbon dioxide/bicarbonate (optional), AST, ALT, lactate dehydrogenase (LDH), total bilirubin, indirect bilirubin, alkaline phosphatase, blood urea nitrogen (BUN)/urea, creatinine, GGT, calcium, phosphorus, glucose, amylase, lipase, total protein, albumin, and uric acid.
- Urinalysis by dipstick analysis: pH, specific gravity, protein, glucose, ketones, blood, leukocyte esterase, and nitrite, with microscopic examination if indicated.
- Pregnancy test (urine or serum) and menstrual history for women of child bearing potential.
- Screening visit only: serum folate, B_{12}

The following laboratory assessments will be performed at the central laboratory according to the laboratory collection recommendations. The sponsor may request additional safety tests be performed based on ongoing data review during the study.

- Iron studies: Serum iron, TIBC, transferrin, soluble transferrin receptor, ferritin, NTBI, and hepcidin.
- nRBCs
- Erythropoietin levels
- Peripheral blood smear
- Hemoglobin electrophoresis, globin chain mRNA
- Urine microalbumin and creatinine
- Bone Biomarkers: serum BSAP and CTX

11.3. Other Safety Assessments

- Physical examination (including optional evaluation of gonadal size in males)

- Vital signs: weight, heart rate, systolic and diastolic blood pressure, respiration rate, and temperature (measured in degrees Celsius). Height is measured only at screening. If at any visit the systolic blood pressure is ≥ 150 mmHg, the diastolic blood pressure is ≥ 95 mmHg, and/or the absolute increase in either measure from baseline is ≥ 20 mmHg, perform one repeat blood pressure assessment after a minimum of 15 minutes.
- 12-lead ECG
- Anti-drug antibody testing
- Abdominal ultrasound should be performed as needed for patients with clinical signs of a change in spleen size or abnormality in the dose escalation cohorts only.

11.4. Pharmacokinetic and Pharmacodynamic Assessments

11.4.1. Pharmacokinetic Assessments

Pharmacokinetic assessment of ACE-536 concentrations will be performed periodically as outlined in Section 2. Blood samples should be drawn and processed on-site for serum collection at the time points specified. Additional details regarding PK collection and processing can be found in the Study Reference Guide.

11.4.2. Pharmacodynamic Evaluations

Pharmacodynamic assessments including hematologic laboratory assessments and transfusion information will be used to determine erythroid response to ACE-536.

An MRI will be performed at selected sites to evaluate liver iron content (all cohorts), EMH mass size (expansion cohort only), and spleen size (expansion cohort only).

A DXA will be performed at selected sites to evaluate total body, lumbar spine and total hip BMD (expansion cohort only).

Leg ulcer(s) size will be assessed in patients with leg ulcers; photographs should be taken to document changes.

A 6MWT will be performed at selected sites (expansion cohort only) for NTD patients.

An abdominal ultrasound of the spleen will be performed to evaluate spleen size for patients without a history of splenectomy in the dose escalation cohorts only.

Biomarkers for iron metabolism will include serum iron, TIBC, transferrin, soluble transferrin receptor, ferritin, NTBI, and hepcidin.

Biomarkers for bone metabolism will include serum CTX (a marker of bone resorption) and BSAP (a marker of bone formation).

Biomarkers related to the TGF-β superfamily, such as GDF15, may be tested in blood (to be determined). The relationship of biomarkers to erythroid response may be investigated.

The relationship of biomarkers to erythroid response may be investigated.

The sponsor may request additional biomarkers for exploratory research purposes only.

Quality of Life tools including but not limited to the FACT-An and SF-36 questionnaires will assessed.

12. STUDY SCHEDULE

Please refer to Section 2 for the schedule of procedures required for each visit. Note that all windows on visits should be determined relative to the date of the previous dose of ACE-536.

12.1. Screening

- Signature of the current IEC approved ICF should occur prior to initiation of any study-specific screening procedures.
- All screening procedures should occur within 28 days prior to Cycle 1 Day 1.
- Screening MRI for liver iron content (all cohorts at selected sites), MRI of the chest and abdomen for EMH masses (expansion cohort only at selected sites), MRI for spleen (if no prior splenectomy; expansion cohort only at selected sites), abdominal ultrasound of the spleen (if no prior splenectomy; dose escalation cohorts only) and ECHO, MUGA or cardiac MRI (all cohorts) can be performed up to 56 days prior to Cycle 1 Day 1. If performed as part of standard of care, it does not need to be repeated.
- Historical hemoglobin values will be collected for 12 weeks prior to Cycle 1 Day 1. Historical transfusion history will be collected for 12 months prior to Cycle 1 Day 1.
- Baseline hemoglobin will be the average of two measurements; one performed within one day prior to Cycle 1 Day 1, and the other performed during the screening period (Day -28 to Day -1).
- Pregnancy test (urine or serum) and menstrual history is required of female patients of child bearing potential only.
- Concomitant medications taken within 28 days prior to Cycle 1 Day 1 will be documented in the CRF.
- Screen failure information will be maintained to document specific information, including but not limited to, reason for failure.
- For the expansion cohort patients only, administration of quality of life questionnaires.
- For blood pressure measurements, if the systolic blood pressure is ≥ 150 mmHg, the diastolic blood pressure is ≥ 95 mmHg, and/or the absolute increase in either measure from baseline is ≥ 20 mmHg, perform one repeat blood pressure assessment after a minimum of 15 minutes.

12.2. Dosing Days and Interim Visits

- For the first dose of ACE-536, dosing should occur after a minimum of 7 days post-transfusion; subsequent doses should not be given within 24 hours of transfusion or planned transfusion.
- Note: For any RBC transfusions received during the study, collect hemoglobin value just prior to transfusion.
- Each TD patient in the expansion cohort will have a baseline "pre-transfusion hemoglobin threshold" which will be calculated based on transfusion history and will be used for determining when to transfuse during the study
- All screening and Cycle 1 Day 1 procedure results required to confirm eligibility must be obtained and reviewed prior to study drug administration. Patient eligibility must be confirmed from these results.
- A patient will be considered enrolled into the study once a multi-digit patient identification number has been assigned to the patient. After assignment of the patient identification number and the completion of the required procedures, study drug administration may occur. Note that the patient dose must be calculated based on the patient's weight on the day of dosing.
- All study procedures on each dosing day must be done prior to administration of study drug.
- Patients should be observed for a minimum of 30 minutes following treatment with ACE-536.
- If a dose delay is required per the patient dose modification rules (see Section 10.8.1), the patient will not be dosed. The patient will return weekly for assessment of hematology results and adverse events until the patient is eligible to receive the next dose of ACE-536.
- The hematology results can be collected up to 24 hours prior to the dosing day. For Cycle 1 Day 1, the hemoglobin should be available to confirm eligibility within one day prior.
- Any non-serious AEs that occur prior to dosing on Cycle 1 Day 1 should be recorded in the Medical History CRF.
- All AEs that occur after dosing on Cycle 1 Day 1 should be recorded in the AE CRF.
- On subsequent dosing days, all AEs and abnormal findings that might require modification of dosing (see Section 10.8) should be reviewed prior to dosing to ensure that the patient is still eligible to receive additional doses of ACE-536.
- Pregnancy test (urine or serum) and menstrual history is required of female patients of child bearing potential only.
- Physical exam on Cycle 1 Day 1 should include an optional evaluation of gonadal size of male patients.

- For blood pressure measurements, if the systolic blood pressure is ≥ 150 mmHg, the diastolic blood pressure is ≥ 95 mmHg, and/or the absolute increase in either measure from baseline is ≥ 20 mmHg, perform one repeat blood pressure assessment after a minimum of 15 minutes.
- Leg ulcer(s) size will be assessed in patients with leg ulcers; photographs should be taken to document changes.

12.3. Day 85

- Day 85 (± 2 days) is the last possible study day that ACE-536 may be administered, regardless of the cycle.
- For the expansion cohort patients only, administration of quality of life questionnaires.
- For blood pressure measurements, if the systolic blood pressure is ≥ 150 mmHg, the diastolic blood pressure is ≥ 95 mmHg, and/or the absolute increase in either measure from baseline is ≥ 20 mmHg, perform one repeat blood pressure assessment after a minimum of 15 minutes.

12.4. End of Treatment Visit

- The end of treatment visit should occur approximately 28 days after the last dose of ACE-536.
- Patients who discontinue treatment early should complete the end of treatment visit at the time of discontinuation.
- Patients in the expansion cohort who discontinue treatment early or do not complete the Day 85 visit should complete the quality of life questionnaires at the end of treatment visit.
- An abdominal ultrasound of the spleen will be performed for patients without a history of splenectomy in the dose escalation cohorts only.
- An MRI will be performed at selected sites to evaluate liver iron content (all cohorts), and an MRI of the chest and abdomen for EMH mass size (expansion cohort only), and spleen size (expansion cohort only).
- A DXA will be performed at selected sites to evaluate total body, lumbar spine and total hip BMD (expansion cohort only).
- A 6MWT will be performed in NTD patients at selected sites (expansion cohort only).
- Leg ulcer(s) size will be assessed in patients with leg ulcers; photographs should be taken to document changes.
- Pregnancy test (urine or serum) and menstrual history will be collected for women of child bearing potential.
- Physical exam should include an optional evaluation of gonadal size of male patients.

- For blood pressure measurements, if the systolic blood pressure is ≥ 150 mmHg, the diastolic blood pressure is ≥ 95 mmHg, and/or the absolute increase in either measure from baseline is ≥ 20 mmHg, perform one repeat blood pressure assessment after a minimum of 15 minutes.

12.5. End of Study Visit

- The end of study visit should occur approximately 28 days after the end of treatment visit.
- Patients who discontinue treatment early should complete the end of study visit approximately 28 days after the end of treatment visit.
- For the expansion cohort patients only, administration of quality of life questionnaires.
- If a patient has a positive anti-drug antibody (ADA) result at the last visit, the patient may be asked to return for additional ADA testing every three months, until a negative result is obtained or the result is considered to be stabilized.
- Menstrual history will be collected for women of child bearing potential; pregnancy test not required.
- Physical exam should include an optional evaluation of gonadal size of male patients.
- For blood pressure measurements, if the systolic blood pressure is ≥ 150 mmHg, the diastolic blood pressure is ≥ 95 mmHg, and/or the absolute increase in either measure from baseline is ≥ 20 mmHg, perform one repeat blood pressure assessment after a minimum of 15 minutes.
- Leg ulcer(s) size will be assessed in patients with leg ulcers; photographs should be taken to document changes.

12.6. Termination of Study

The sponsor may terminate this study or discontinue a cohort, after consultation with the investigator(s), or at any time, for safety or administrative reasons. The sponsor will terminate the study if the occurrence of SAEs or other findings suggests unacceptable risk to the health of the patients.

13. STUDY DRUG MATERIALS AND MANAGEMENT

13.1. Study Drug

ACE-536 is a recombinant fusion protein consisting of a modified form of the extracellular domain (ECD) of the human activin receptor IIB (ActRIIB) linked to the human IgG1 Fc domain.

13.2. Study Drug Packaging and Labeling

ACE-536 drug product is provided as a sterile, liquid formulation at a nominal concentration of 50 mg/mL in Tris-buffered saline, pH 7.2 ± 0.5, contained in type I, 13-mm borosilicate glass vials (stoppered and crimp-sealed). Each single-use vial contains 0.5 mL (25 mg) of ACE-536 solution for injection.

13.3. Study Drug Storage

ACE-536 is stored frozen at ≤ -65˚C until use.

13.4. Study Drug Preparation

Please refer to the Study Reference Guide, provided under separate cover, for detailed ACE-536 drug handling, administration, and storage instructions.

13.5. Study Drug Administration

ACE-536 will be administered by SC injection. Multiple injections may be required to administer the appropriate dose at higher dose levels; however, no more than 3 injections will be administered per dose. Patients should be observed for a minimum of 30 minutes following treatment with ACE-536.

13.6. Study Drug Accountability

Accountability for ACE-536 is the responsibility of the investigator. Investigational clinical supplies must be received by a designated person at the clinical site and kept in a secured location. The clinical site must maintain accurate records demonstrating dates and amounts of ACE-536 received, to whom it was dispensed (patient-by-patient accounting), and accounts of any ACE-536 accidentally or deliberately destroyed or returned.

Unless otherwise notified, all vials of ACE-536, both used and unused, must be saved for drug accountability purposes. The used vials may be discarded, per the institution's standard practice, after drug accountability assessment has been completed by the clinical monitor. At the end of the study, the sponsor will provide direction for the outcome of all unused vials. Following on the sponsor's instructions, the investigator must either return all unused vials of ACE-536 to the sponsor or destroy them at the clinical site. In either case, the outcome must be documented on the drug accountability log.

13.7. Study Drug Handling and Disposal

Please refer to the Study Reference Guide, provided under separate cover, for detailed ACE-536 drug handling, administration, storage, and disposal instructions.

14. ASSESSMENT OF SAFETY

14.1. Adverse Event Definitions

Adverse Event

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

Abnormal laboratory and other abnormal investigational findings (e.g., physical exam, ECG) should not be reported as AEs unless they are associated with clinical signs and symptoms, lead to treatment discontinuation, or are otherwise considered clinically relevant by the investigator. In cases of surgical or diagnostic procedures, the condition/illness leading to such a procedure is considered as the AE rather than the procedure itself. In case of a fatality, the cause of death is considered as the AE, and the death is considered as its outcome.

Unexpected Adverse Events

An unexpected AE is an AE that is not described in nature or severity in the Investigator's Brochure.

Events Not to Be Considered as Adverse Events

Pre-existing medical conditions/signs/symptoms present before the Screening period that do not worsen in severity or frequency during the study are defined as Baseline Medical Conditions, and are not to be considered AEs. Anticipated day-to-day fluctuations of pre-existing conditions, including the disease under study, that do not represent a clinically significant exacerbation or worsening need not be considered adverse events.

Serious Adverse Event

A serious adverse event (SAE) is any AE, occurring at any dose level/regimen and regardless of causality that:

- Results in death.
- Is life-threatening: Life-threatening means that the patient was at immediate risk of death from the reaction as it occurred (i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form).
- Requires inpatient hospitalization or prolongation of existing hospitalization; however, a hospitalization for an elective procedure will not be considered an SAE.
- Results in persistent or significant disability/incapacity.

- Is a congenital anomaly/birth defect.
- Is an important medical event: an important medical event is an event that may not result in death, be life-threatening, or require hospitalization, but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions for SAEs. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

For the purposes of reporting, any suspected transmission of an infectious agent via a medicinal product is also considered a serious adverse reaction and all such cases should be reported in expedited manner.

Events Not to Be Considered as Serious Adverse Events

Elective hospitalizations to administer or to simplify study treatment or procedures are not considered SAEs.

14.2. Pregnancy and In Utero Drug Exposure

The investigator will attempt to collect pregnancy information if a female patient or a male patient's female partner becomes pregnant while the patient is participating in this study. The pregnancy information will be recorded on the appropriate form and must be submitted to the Sponsor within 2 weeks of learning of the pregnancy. The patient or partner will be followed for the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor or designee. Generally, follow up will be no longer than 6 to 8 weeks following the estimated delivery date. Any premature termination of the pregnancy will be reported.

14.3. Severity

Investigators must evaluate the severity/intensity of AEs according to the current active minor version of the National Cancer Institute Common Toxicity Criteria for Adverse Events, version 4.0 (NCI-CTCAE v4.0), preferentially using the graded scales. If there is a change in severity of an adverse event, it must be recorded as a separate event. If a particular AE's severity/intensity is not specifically graded, the investigator should apply the general guidelines for determination of Grade 1 through Grade 5 as listed in the NCI-CTCAE v4.0 cover page (as shown below), using their best medical judgment:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.).

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living

(bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE.

14.4. Relationship to Study Drug

Investigators must also assess the causal relationship of each AE to ACE-536. Factors for the assessment of causal relationship include, but are not limited to, temporal relationship between the AE and the administration of ACE-536, known side effects of ACE-536, medical history, concomitant therapy, course of the underlying disease, and pertinent study procedures.

Probable: A causal relationship is clinically/biologically highly plausible and there is a plausible time sequence between onset of the AE and administration of ACE-536 and there is a reasonable response on withdrawal.

Possible: A causal relationship is clinically/biologically plausible and there is a plausible time sequence between onset of the AE and administration of ACE-536.

Unlikely: A causal relationship is improbable and another documented cause of the AE is most plausible.

Unrelated: A causal relationship can be definitively excluded and another documented cause of the AE is most plausible.

14.5. Documentation and Methods of Reporting of Adverse Events by Investigator

It is the responsibility of the Investigator to document all adverse events that occur during the study. Patients will be evaluated and questioned generally for AEs during the course of the study, starting at the signing of the informed consent. The Investigator must report in detail all adverse signs and symptoms which are either volunteered by subjects or observed during or following the course of investigational product administration on the appropriate CRF page. All non-serious AEs occurring after signing of the ICF until a patient is dosed on Cycle 1 Day 1 are to be documented on the medical history CRF. All AEs and SAEs occurring after the Cycle 1 Day 1 dose through 56 days after the last study drug administration (End of Study visit) are to be reported and documented on the AE CRF.

All AEs spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures will be recorded on the AE CRF. Any clinically relevant changes in laboratory assessments, or other clinical findings as described in Section 14.1, are considered AEs and must be recorded on the AE CRF. AEs are to be followed for resolution as described in Section 14.6.

It is important that each AE report include a description of the event, duration (onset and resolution dates), severity, relationship with ACE-536, any other potential causal factors, any treatment given or other action taken (including dose modification or discontinuation of ACE-536) and outcome. In addition, serious AEs (SAEs) should be identified and the

appropriate seriousness criteria documented. AEs categorized as SAEs must also be documented using an SAE Report Form as described in Section 14.5.1.

Specific guidance can be found in the CRF Completion Guidelines provided by the Sponsor or designee.

14.5.1. Documentation of Serious Adverse Events

All SAEs that occur after the first study drug administration on Cycle 1 Day 1 until 56 days after the last study drug administration (End of Study visit) are to be documented on the AE CRF. SAEs should not be reported for patients who are considered screen failures unless the event is deemed due to a protocol required procedure.

For all SAEs, an SAE form must be completed with as much information as possible and submitted within the time frame described in Section 14.7.

When new significant information is obtained, as well as when the outcome of an event is known, the investigator should record the information on a new SAE form. If the patient was hospitalized, a copy of the discharge summary must be included as part of the patient medical file. In all instances, the investigator should follow up with patients until the outcome of the SAE is known.

14.6. Reporting Period and Monitoring of Patients with Adverse Events

As described in Section 14.5, all AEs must be recorded in the CRF up until the end of the treatment period. All patients who took at least one dose of study drug, whether they completed the treatment period or not, should complete the end of treatment procedures.

All AEs will be followed until clinical database lock (or resolution if it occurs before database lock). All SAEs will undergo active follow up until resolved or the event becomes chronic or stable. Follow up data for SAEs obtained after clinical database lock will be incorporated into the ACE-536 safety database.

14.7. Notification about Serious Adverse Events

If an SAE occurs during the reporting period, the investigator must immediately (i.e., within a maximum 24 hours after becoming aware of the event) inform the sponsor via the CRO by telephone, fax, or e-mail.

All written reports should be transmitted using the study-specific SAE Report Form, which must be completed by the investigator following specific completion instructions. Names, addresses, and telephone and fax numbers for SAE reporting are located on the SAE Report Form and in the completion instructions provided in the Study Manual. When an SAE (or follow up information) is reported by telephone, a written report must be sent immediately thereafter by fax or e-mail. Reporting procedures and timelines for follow up information are the same as for the initially reported SAE.

Relevant pages from the CRF may be provided in parallel (e.g., medical history, concomitant therapy). In all cases, the information provided in the SAE Report Form must be consistent with the data that are recorded in the corresponding sections of the CRF.

The investigator/reporter must respond to any request for follow up information or to any question the Sponsor or designee may have on the AE within the same timelines as described for initial reports. This is necessary to permit a prompt assessment of the event by the Sponsor and (as applicable) to allow the Sponsor to meet regulatory timelines associated with expedited reporting obligations.

Requests for follow up will usually be made by the responsible clinical research associate or Medical Monitor, or an Acceleron pharmacovigilance representative who may contact the investigator directly to obtain clarification on a particularly critical event.

14.7.1. Safety Reporting to Health Authorities, Independent Ethics Committees, Institutional Review Boards, and Investigators

The Sponsor will send appropriate safety notifications to Health Authorities in accordance with applicable laws and regulations.

The investigator must comply with any applicable site-specific requirements related to the reporting of SAEs involving his/her patients to the IEC that approved the study.

In accordance with International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines, the Sponsor will inform the investigator of "findings that could adversely affect the safety of patients, impact the conduct of the study, or alter the IEC's approval/favorable opinion to continue the study."

The Sponsor will inform the investigator of AEs that are both serious and unexpected and are considered to be related to ACE-536 ("suspected unexpected serious adverse reactions" or SUSARs). The investigator should place copies of these Safety Reports in the Investigator Site File. National regulations with regard to Safety Report notifications to investigators will be followed.

When specifically required by regulations and guidelines, the Sponsor will provide appropriate Safety Reports directly to the concerned lead IEC and will maintain records of these notifications. When direct reporting by the Sponsor is not clearly defined by national or site-specific regulations, the investigator will be responsible for promptly notifying the concerned IEC of any Safety Reports and for filing copies of all related correspondence in the Investigator Site File.

For studies covered by the European Union Clinical Trials Directive 2001/20/EC, the Sponsor's responsibilities regarding the reporting of SAEs/SUSARs will be carried out in accordance with that Directive and with the related Detailed Guidances.

15. STATISTICS

15.1. Analysis Populations

For all analysis populations, patients will be analyzed according to assigned cohort.

The Intent-to-Treat (ITT) Population: All patients enrolled in the study.

Efficacy Evaluable (EE) Population: All patients administered at least one dose of ACE-536, and 1) at least 2 hemoglobin values ≥ 14 days apart post-treatment, not influenced by transfusion in non-transfusion dependent patients, OR, 2) at least 12 weeks of transfusion frequency data prior to Cycle 1 Day 1 and following Cycle 1 Day 1 in transfusion dependent patients.

Safety Population: All patients who received at least 1 dose of ACE-536.

Pharmacokinetics Population: All patients who have received at least 1 dose of ACE-536 and have sufficient pharmacokinetic samples collected and assayed for PK analysis.

15.2. Statistical Plan

Summary statistics will be presented for continuous/quantitative variables, by way of number of patients (n), mean, standard deviation (SD), median, minimum and maximum, and by way of group frequencies and percentages for categories of qualitative variables. Percentages will be calculated using the total patients per treatment/subgroup. 95% confidence intervals (CIs) will be presented as applicable.

All patient data will be presented in separate data listings.

15.2.1. Patient Accountability and Demographics

Exposure to study drug and reasons for discontinuation of study will be tabulated, and demographics will be presented using descriptive statistics (i.e., mean, standard deviation, median, and range).

15.2.2. Primary Efficacy Analysis

The primary efficacy endpoint is defined as the proportion of β-thalassemia patients who have an erythroid response, defined as 1) a hemoglobin increase of ≥ 1.5 g/dL from baseline for ≥ 14 days (in the absence of RBC transfusions) in non-transfusion dependent patients or 2) ≥ 20% reduction in RBC transfusion burden compared to pretreatment in transfusion dependent patients.

Transfusion burden will be defined as the ratio of RBC transfusion units (or mLs) transfused during an interval to the duration (days) of that interval, where the interval may be during the pretreatment period or the treatment plus follow-up period. The interval during the pretreatment period will be defined as the 12 weeks prior to Cycle 1 Day 1. An interval during the treatment plus follow-up period will be defined as any 12-week interval after Cycle 1 Day 1.

The erythroid response will be summarized using both a point estimate and its exact 95% confidence interval based on binomial distribution. The primary efficacy analysis will be performed using the EE population.

No direct comparison testing with concurrent or historical controls will be performed.

Baseline hemoglobin will be an average of 2 or more measurements; one measure performed within one day prior to Cycle 1 Day 1 and at least one other performed during the screening period (Day -28 to Day -1). Hemoglobin measurements within 2 weeks following RBC transfusion will be excluded.

15.2.3. Secondary Analysis

Safety analysis: To assess clinical safety, adverse events, vital sign measurements, and clinical laboratory information will be summarized. Adverse Events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Changes from baseline for clinical laboratory values and vital signs will be summarized over time. Descriptive statistics and shift tables will be generated as appropriate.

The **secondary efficacy/pharmacodynamic analysis** will be performed using the ITT and EE populations and will include:

- Erythroid response, as defined above for primary endpoint, performed using the ITT population.
- For non-transfusion dependent patients, the proportion of patients with an increase of ≥ 1.0 g/dL from pretreatment hemoglobin maintained ≥ 8 weeks.
- For non-transfusion dependent patients, the proportion of patients with an increase of ≥ 1.5 g/dL from pretreatment hemoglobin maintained ≥ 8 weeks.
- For transfusion dependent patients, the proportion of patients who have ≥ 50% reduction in RBC transfusion burden compared to pretreatment using 12-week intervals.
- For transfusion dependent patients, the proportion of patients who have ≥ 50% reduction in RBC transfusion burden compared to pretreatment using 8-week intervals.
- For transfusion dependent patients, the proportion of patients who have no RBC transfusions ≥ 8 weeks.
- Time to erythroid response and duration of erythroid response.
- Change from pretreatment in transfusion burden in transfusion dependent patients
- Change in hemoglobin level in non-transfusion dependent patients
- Change in pre-transfusion hemoglobin levels in transfusion dependent patients
- Change from baseline in erythropoiesis parameters including serum erythropoietin levels, hemoglobin analysis (electrophoresis, globin chain RNA), reticulocytes, and nucleated RBCs
- Change from baseline in hemolysis parameters include haptoglobin, indirect bilirubin, and lactate dehydrogenase (LDH)
- Change from baseline in iron metabolism parameters include serum iron, total iron binding capacity (TIBC), transferrin, calculated transferrin saturation, soluble

transferrin receptor, ferritin, non-transferrin bound iron (NTBI), hepcidin, and liver iron content (LIC) by MRI

- Change from baseline in bone metabolism parameters include bone specific alkaline phosphatase (BSAP) and C-telopeptide of type I collagen (CTX)
- Change from baseline in spleen size (in subset of patients with no prior-splenectomy)

The **exploratory endpoints** will include evaluation of biomarkers related to the TGF-β superfamily, quality of life, change in spleen size (in subset of patients with no prior splenectomy), change in BMD, change in EMH mass size, change in leg ulcer size, and change in the 6MWT distance in NTD patients.

All binary endpoints will be summarized using both a point estimate and its exact confidence interval based on the binomial distribution. The time-to-event type secondary endpoints will be analyzed using Kaplan-Meier method to estimate the survival curve and median time to event and 95% confidence interval.

15.2.4. Pharmacokinetics analysis

Non-compartmental PK parameters for ACE-536, such as maximum plasma concentration (C_{max}), time to maximum plasma concentration (t_{max}), and area under the concentration/time curve (AUC), will be estimated. Dose proportionality may be assessed using the exposure data (eg, C_{max}, AUC) after the first dose if the sufficient dose levels are studied. Descriptive statistics will be provided for serum concentrations and PK parameters. The relationship between ACE-536 exposure and response (ie, safety, efficacy, and biomarkers) may be explored, if appropriate.

15.2.5. Anti-drug antibody analysis

The results of anti-drug and neutralizing antibody testing for ACE-536 and human ActRIIB protein versus time will be presented. Exploratory analysis will be performed on the potential effect of anti-drug antibodies on ACE-536 PK and drug exposure if anti-drug antibody tests are deemed positive.

15.3. Determination of Sample Size

There is no formal sample size calculation for the dose escalation portion of the study. A sample size of 30 evaluable patients in the expansion cohort will provide approximately 87% power with 1-sided significance level of 0.05 to differentiate an erythroid response rate of 30% from a minimal erythroid response rate of 10%.

15.4. Interim Analysis

There are no planned interim analyses. However, safety and erythroid response data will be reviewed periodically throughout the study.

15.5. Deviation from Original Analysis Plan

A formal Statistical Analysis Plan (SAP) for the analysis and presentation of data from this study will be prepared before the database lock. Deviations from the statistical analyses outlined in this protocol will be indicated in this plan; any further modifications will be noted in the final clinical study report.

16. SOURCE DOCUMENTATION AND INVESTIGATOR FILES

16.1. Study Monitoring

The clinical monitor will arrange to visit the clinical sites at regular intervals during the study. The monitoring visits must be conducted according to the applicable ICH and GCP guidelines to ensure protocol adherence, quality of data, drug accountability, compliance with regulatory requirements, and continued adequacy of the clinical sites and their facilities. During these visits, CRFs and other data related to the study will be reviewed and any discrepancies or omissions will be resolved. The clinical monitor will be given access to study-relevant source documents (including medical records) for purposes of source data verification.

16.2. Audits and Inspections

The investigators and clinical sites will permit trial-related monitoring, audits, IEC review, and regulatory inspections as requested by FDA or other health authorities and the sponsor or designee. In addition to CRFs, the clinical site will permit direct access to source data/documents (ie, original medical records, laboratory reports, hospital documents, progress reports, signed ICFs, etc.). During and/or after completion of the study, quality assurance officers named by the sponsor or the regulatory authorities may wish to perform on-site audits. The investigator is expected to cooperate with any audit and provide assistance and documentation (including source data) as requested.

17. QUALITY CONTROL AND QUALITY ASSURANCE

17.1. Data Quality Control and Quality Assurance

17.1.1. Investigator Responsibility

The investigator is responsible for ensuring the study is conducted according to the protocol, Code of Federal Regulations, GCP, and applicable regulatory requirements. The investigator's responsibilities are outlined in these documents and must include the responsibility to obtain a signed informed consent prior to patient participation in the study.

17.1.2. Protocol Modifications

The investigator should not modify the protocol without agreement from the sponsor and prior review or approval by the IEC, unless an emergency situation requires protocol modification to ensure the safety of patients. Any deviations from the protocol should be documented by the investigator or designee.

18. CONFIDENTIALITY

To maintain patient privacy, all CRFs, study drug accountability records, study reports and communications will identify the patient by the assigned patient identification number. The investigator will grant clinical monitor(s) and auditor(s) from the sponsor or designee and regulatory authorities' access to the patient's original medical records for verification of data gathered on the CRFs and to audit the data collection process. The patient's confidentiality will be maintained and will not be made publicly available. The patient's medical information will only be released to the extent permitted by the applicable laws and regulations.

All information regarding the investigational product supplied by the sponsor to the investigator is privileged and confidential information. The investigator agrees to use this information to accomplish the study and will not use it for other purposes without consent from the sponsor. It is understood that there is an obligation to provide the sponsor with complete data obtained during the study. The information obtained from the clinical study will be used towards the development of the investigational product and may be disclosed to regulatory authorities, other investigators, corporate partners, or consultants as required.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by United States Law. This Web site will not include information that can identify patients. At most, the web site may include a summary of the results.

Publication Policy

All information concerning ACE-536 is considered confidential and shall remain the sole property of the sponsor. The investigator(s) agree to use this information only in conducting the study and shall not use it for any other purposes without the sponsor's written approval. The investigator(s) agree not to disclose the sponsor's confidential information to anyone except to persons involved in the study that need such information to assist in conducting the study, and then only on like terms of confidentiality and non-use.

It is understood by the investigator(s) that the information developed from this clinical study will be used by the sponsor in connection with the development of ACE-536, and therefore may be disclosed as required to regulatory agencies. To allow for the use of the information derived from clinical studies, it is understood that there is an obligation to provide the sponsor with complete test results and all data developed in the study.

No publication or disclosure of study results will be permitted except as specified in a separate, written, agreement between the sponsor and the investigator(s).

19. PROTOCOL AMENDMENTS

Protocol amendments that impact patient safety, change the scope of the investigation, or affect the scientific quality of the study must be approved by the IEC and submitted to the appropriate regulatory authorities before implementation.

In the event that the protocol needs to be modified immediately to eliminate an apparent hazard to a patient, the sponsor will implement the protocol change and subsequently amend the protocol and notify the regulatory authorities and/or the IEC, as appropriate.

20. DATA HANDLING AND RECORDKEEPING

20.1. Case Report Form Completion

Case report forms will be completed for each enrolled patient. It is the investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the patient's CRF. Source documentation supporting the CRF data should indicate the patient's participation in the study and should document the dates and details of study procedures, AEs, and patient status.

Investigators will maintain copies of the CRFs at the clinical site. For patients who discontinue or terminate from the study, the CRFs will be completed as much as possible, and the reason for the discontinuation or termination clearly and concisely specified on the appropriate CRF.

20.2. Retention of Records

The investigator will maintain all study records according to ICH-GCP and applicable regulatory requirements. Records will be retained for at least 2 years after the last marketing application approval or 2 years after formal discontinuation of the clinical development of the investigational product, or according to applicable regulatory requirements. If the investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility. The sponsor must be notified in writing if a custodial change occurs.

21. STUDY FINANCE AND INSURANCE

21.1. Study Finance

The costs necessary to perform the study will be agreed with each Investigator and will be documented in a separate financial agreement that will be signed by the Investigator and Acceleron Pharma Inc. or designee, prior to the study commencing.

21.2. Insurance

The Sponsor has insurance coverage for study-related ACE-536 induced injury and other liabilities incurred during clinical studies which will provide compensation for any study-related injury according to the guidelines set out by the Association of the British Pharmaceutical Industry (ABPI), namely “Clinical Studies Compensation for Medicine Induced Injury”.

22. REFERENCES

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23. APPENDICES

23.1. Appendix 1: New York Heart Association Classification - The Stages of Heart Failure[9]

Class 1 – Class 1 heart failure – patients with cardiac disease but resulting in no limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.
Class 2 – Class 2 heart failure – patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.
Class 3 – Class 3 heart failure – patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.
Class 4 – Class 4 heart failure – patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort increases.

23.2. Appendix 2: National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE)

See http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE