Official Protocol Title:	An Open-Label Extension Study to Evaluate the Long-Term Effects of ACE-536 in Patients with β-Thalassemia Previously Enrolled in Study A536-04
NCT number:	NCT02268409
Document Date:	29-Mar-2019

CLINICAL STUDY PROTOCOL

An Open-Label Extension Study to Evaluate the Long-Term Effects of ACE-536 in Patients with β-Thalassemia Previously Enrolled in Study A536-04

INVESTIGATIONAL PRODUCT:	**Luspatercept (ACE-536)**
PROTOCOL NUMBER:	**A536-06**
EUDRACT NUMBER:	**2014-001281-94**
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:	**14-Apr-2014**
AMENDMENT 01 DATE:	**08-Dec-2014**
AMENDMENT 02 DATE:	**03-Jun-2015**
AMENDMENT 03 DATE:	**23-May-2016**
AMENDMENT 04 DATE:	**16-Aug-2016**
AMENDMENT 05 DATE:	**12-Sept-2017**
AMENDMENT 06 DATE:	**29-Mar-2019**

ACCELERON PHARMA SIGNATURE PAGE

Signature: 

Date: 02-APR-2019
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:** _____
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 International Conference of Harmonization (ICH) Good Clinical Practices (GCP) 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, ICH Guidelines, 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 FDA regulations, ICH Guidelines, 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 Direct Line: PPD Mobile: PPD Fax: PPD PPD
Chiltern Medical Advisor	PPD	Chiltern Via M. Nizzoli, 6 Milano 20147 Italia Direct Line: PPD Mobile: PPD Fax: PPD PPD
Pharmacovigilance	Chiltern	PPD PPD PPD PPD PPD 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	
Title of Study: An Open-Label Extension Study to Evaluate the Long-Term Effects of ACE-536 in Patients with β-Thalassemia Previously Enrolled in Study A536-04	
Study Centers: up to 15	
Phase of Development: 2	

Objectives:

Primary:

- To evaluate the long-term safety and tolerability of ACE-536 in patients with β-thalassemia who were previously enrolled in study A536-04.

Secondary:

- To evaluate erythroid response defined as the proportion of patients with:
  - Mean hemoglobin increase ≥ 1.5 g/dL over a continuous 12-week interval compared to baseline in non-transfusion dependent (NTD) patients, not influenced by red blood cell (RBC) transfusion, OR
  - Reduction in RBC transfusion burden by ≥ 50% over a continuous 12-week interval compared to the 12 weeks prior to the start of treatment in transfusion dependent (TD) patients.
- To evaluate erythroid response defined as proportion of patients with:
  - Mean hemoglobin increase ≥ 1.5 g/dL over a continuous 8-week interval compared to baseline in NTD patients, not influenced by RBC transfusion, OR
  - Reduction in RBC transfusion burden by ≥ 20% over a continuous 8-week interval compared to the 8 weeks prior to the start of treatment in TD patients.
- To evaluate the time to erythroid response and duration of erythroid response.
- To evaluate the mean change from baseline over an 8- or 12-week period in hemoglobin level in NTD patients not influenced by RBC transfusion
- To evaluate the mean % change from baseline in transfusion burden over an 8- or 12-week period in TD patients.
- To evaluate the mean change in pre-transfusion hemoglobin levels in TD patients
- To evaluate changes in markers of erythropoiesis, hemolysis, iron overload, and iron metabolism

- To examine the pharmacokinetic (PK) profile of ACE-536 in patients with β-thalassemia

Exploratory:

- To evaluate biomarkers related to the transforming growth factor beta (TGF-β) superfamily
- To evaluate patient self-reported quality of life using tools including but not limited to the Functional Assessment of Cancer Therapy-Anemia Scale (FACT-An) and Short Form (36) Health Survey (SF-36) questionnaires
- To evaluate change in extramedullary hematopoiesis (EMH) mass size by MRI
- To evaluate change in spleen size by MRI
- To evaluate change in bone mineral density (BMD) by DXA
- To evaluate change in leg ulcers
- To evaluate change in the 6-minute walk test (6MWT) distance in NTD patients

Methodology

Study Design:

This open-label extension study will evaluate the effects of up to 60 months of ACE-536 treatment in patients with β-thalassemia previously enrolled and treated with ACE-536 for up to 3 months in study A536-04. The base study (A536-04) is a phase 2, open-label, ascending dose study to evaluate the effects of ACE-536 in patients with β-thalassemia. A total of up to 64 patients may be enrolled in study A536-04 and may be eligible for study A536-06.

Consenting patients that meet the A536-06 eligibility criteria may immediately roll over from A536-04 to study A536-06 following the last ACE-536 dose. These patients may forego the End of Study (EOS) visit in study A536-04 to begin study A536-06. For these patients, Cycle 1 Day 1 (C1D1) of study A536-06 may take place 28 (± 7) days after the last dose administered in study A536-04, which may coincide with the patient's A536-04 End of Treatment (EOT) visit. These patients are considered "patients without treatment interruption".

Patients who complete the EOS visit for study A536-04 and are ≥ 28 days post EOS visit are considered "patients with treatment interruption" and will be re-assessed for eligibility by meeting all eligibility criteria plus additional inclusion criteria, as defined in Section 9.3.

Patients who do not meet the above criteria (e.g., > 35 days after last dose in study A536-04 and < 28 days post EOS visit in study A536-04) may still participate, but should be treated as patients with treatment interruption and should not begin study A536-06 C1D1 until they have reached ≥ 28 days post EOS visit from study A536-04 so that new baseline assessments can be measured.

For patients without treatment interruption, transfusion status (NTD or TD) will carry-over from the base study A536-04. For NTD patients with treatment interruption, transfusion status will be reassessed prior to C1D1 of study A536-06. NTD patients with treatment interruption are defined as patients who require transfusion of < 4 units of RBCs over the 8 weeks prior to Cycle 1 Day 1 of study A536-06. For TD patients with treatment interruption, transfusion status will carry-over from the base study A536-04.

A patient without treatment interruption may continue to be dosed with ACE-536 at the same dose level (rounded to nearest A536-06 starting dose level) administered at their last dose in

study A536-04 (unless a dose reduction is required based upon patient dose modification rules from study A536-06). All patients with treatment interruption will be initially treated with ACE-536 at a starting dose level of 0.8 mg/kg which has been determined to be safe and well tolerated by the Safety Review Team (SRT) based on data from study A536-04.

Each TD patient 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 of base study A536-04 (for patients without treatment interruption) or study A536-06 (for patients with treatment interruption). During treatment, if the pre-transfusion hemoglobin level 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).

Patients will participate in the extension study A536-06 for up to 97 months, including a 28-day (1 month) screening period, a 60-month treatment period and a 3-year (36 months) follow-up period.

The SRT established for study A536-04 will review safety data and pharmacodynamic (PD) data periodically throughout study A536-06.

Starting Dose Levels

A patient without treatment interruption may continue to be dosed with ACE-536 at the same dose level (rounded to nearest A536-06 starting dose level) administered at their last dose in study A536-04 (unless a dose reduction is required based upon patient dose modification rules from study A536-06). All patients with treatment interruption will be initially treated with ACE-536 at a starting dose level of 0.8 mg/kg which has been determined to be safe and well tolerated by the Safety Review Team (SRT) based on data from study A536-04. Examples of possible starting dose levels with dose modifications (reductions and titrations) are below for reference.

Examples of Possible Starting Dose with Dose Modifications (Reductions and Titrations)

3rd Dose Reduction	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	1.25 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		

- Starting dose level for TD and NTD patients to be determined based on data from study A536-04 (range: 0.2-1.25 mg/kg).
- Starting dose level for patients without treatment interruption will be last dose level (rounded to nearest A536-06 starting dose level) administered in study A536-04 unless a dose reduction is required based upon patient dose modification rules from study A536-06.
- Patients may be titrated up and down dose levels as required per protocol to meet dose modification and titration rules.
- Patients who require more than 2 dose reductions due to an AE should be discontinued from treatment and complete the EOT and EOS visits.
- The maximum dose titration will not exceed the maximum dose level tested in base study A536-04.

Individual Dose Modification Rules

The following dose modification rules include both PD and safety parameters which may require a dose delay and/or a dose reduction. These rules should be assessed prior to every dosing visit for all patients. If a dose delay is required, the patient should have weekly visits to assess hematology results and adverse events (AE) until the patient is eligible to receive the next dose of ACE-536.

Observation on Dosing Day	Action	ACE-536 Dose Modification
Hemoglobin ≥ 13 g/dL	Hold dose and monitor patient weekly until hemoglobin < 12 g/dL	Resume dosing once hemoglobin < 12 g/dL at the same dose level
Hemoglobin increase ≥ 2 g/dL from the previous dosing day (not attributable to RBC transfusion)	Continue dosing	Reduce the dose by 1 dose level
Non-hematologic AE ≥ Grade 3, or hematologic AE ≥ 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 the dose by 1 dose level[a]
Grade 3 leukocytosis (WBC >100,000/μL)		Discontinue treatment

[a] Patients who require more than 2 dose reductions due to an AE should be discontinued from treatment and complete the EOT, PTFU, LTFU, and EOS visits.

Individual Dose Titration for NTD Patients

For all NTD patients (with and without treatment interruption), the dose level will be assessed for titration at Day 1 of every cycle starting at C3D1 (e.g., C3D1, C4D1, C5D1, etc.). The dose level should be titrated individually for each patient not to exceed the maximum dose level evaluated in the base study A536-04 as follows:

- If hemoglobin increase from baseline is < 1.5 g/dL throughout the previous two 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 two consecutive measurements during the previous two 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 two consecutive measurements during the previous two 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 two or more measurements performed during the screening period for base study A536-04 (for patients without treatment interruption) or study A536-06 (for patients with treatment interruption). Hemoglobin measurements within 2 weeks following RBC transfusion will be excluded from both the calculation of the mean baseline hemoglobin and for evaluation of dose titration for a patient during treatment.

Individual Dose Titration for TD Patients

For all TD patients (with and without treatment interruption), the dose level will be assessed for titration at Day 1 of every cycle starting at C3D1 (e.g., C3D1, C4D1, C5D1, etc.). The dose

level should be titrated individually for each patient not to exceed the maximum dose level evaluated in the base study A536-04 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).

Number of patients: A planned total of up to 64 patients who enrolled in base study A536-04 may be eligible for the extension study A536-06.

Inclusion Criteria:

All patients must meet the following criteria

1. Completion of the treatment period in the base study A536-04.
2. 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.
3. Patient is able to adhere to the study visit schedule, understand and comply with all protocol requirements.
4. Patient understands and is able to provide written informed consent.

Patients with treatment interruption (defined as patients who complete the EOS visit for study A536-04 and are ≥ 28 days post EOS visit) must also meet the following criteria

5. Mean hemoglobin concentration < 10.0 g/dL of 2 measurements (not influenced by RBC transfusion) (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 NTD patients.
6. Adequate folate levels or on folate therapy.
7. Platelet count ≥ 100 x 10^9/L and ≤ 1,000 x 10^9/L.
8. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) < 3 x upper limit of normal (ULN).
9. Serum creatinine ≤ 1.5 x ULN.
10. Ejection fraction ≥ 50% by Echocardiogram (ECHO) or Multi gated acquisition scan (MUGA).

Exclusion Criteria:

All patients must not meet any of the following criteria

1. Discontinuation/withdrawal from study A536-04 due to patient request, patient unwillingness or inability to comply with the protocol, pregnancy, use of prohibited

medication (e.g., hydroxyurea), medical reason or AE, hypersensitivity reaction to the study drug, at the discretion of the sponsor, or loss to follow-up prior to completion of the treatment period.

2. 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.
3. Symptomatic splenomegaly.
4. Splenectomy within 56 days prior to Cycle 1 Day 1.
5. 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.
6. Patients receiving or planning to receive hydroxyurea treatment. Patients must not have had hydroxyurea within 90 days of Cycle 1 Day 1.
7. For patients with treatment interruption: Iron chelation therapy if initiated within 56 days prior to Cycle 1 Day 1.
8. 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 and low molecular weight (LMW) heparin for superficial vein thrombosis (SVT) is permitted).
9. Treatment with another investigational drug (including sotatercept [ACE-011]) 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 at any time between the end of treatment of the base study A536-04 and Cycle 1 Day 1.
10. Known positive for human immunodeficiency virus (HIV), active infectious hepatitis B (HBV) or active infectious hepatitis C (HCV).
11. Uncontrolled hypertension defined as systolic blood pressure (SBP) ≥ 150 mm Hg or diastolic blood pressure (DBP) ≥ 100 mm Hg.
12. 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).
13. Pregnant or lactating females.
14. History of severe allergic or anaphylactic reactions or hypersensitivity to recombinant proteins or excipients in the investigational drug.
15. Any other condition not specifically noted above which, in the judgment of the investigator, would preclude the patient from participating in the study.

Investigational product, dosage and mode of administration:

ACE-536 drug product will be provided as either a sterile liquid formulation or a lyophilized powder formulation for reconstitution with water. Each single-use vial of the liquid formulation contains 25 mg of ACE-536 in a 0.5 mL Tris-buffered saline solution (50 mg/mL). Each single-use vial of the lyophilic formulation may contain either 25 mg, 50 mg, or 75 mg ACE-536 for reconstitution with 0.5 mL, 1 mL, or 1.5 mL water, respectively, to form a citrate-buffered solution (50 mg/mL) for injection.

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

Criteria for Evaluation

Safety Assessments:

- All patients will be assessed for safety by monitoring AEs, clinical laboratory tests, vital signs, electrocardiogram (ECG), anti-drug antibody (ADA) testing, abdominal MRI/ultrasound and physical examination.

Efficacy Assessments:

- Patients will be assessed for erythroid response over a 62-month period following initiation of treatment. Erythroid response endpoints will be determined by monitoring hematologic laboratory values and RBC transfusions.
- Other secondary efficacy endpoints will be assessed by examining other hematology, erythropoiesis, hemolysis, iron overload, and iron metabolism parameters.
    - Erythropoiesis parameters may include serum erythropoietin (EPO) levels, reticulocytes, nucleated RBCs and soluble transferrin receptor
    - Iron overload/metabolism parameters may include serum iron, total iron binding capacity (TIBC), transferrin, soluble transferrin receptor, calculated transferrin saturation, ferritin, non-transferrin bound iron (NTBI), hepcidin, Liver iron content (LIC), and iron chelation therapy use.

PK Assessments:

- Blood samples will be drawn to evaluate PK parameters.

Exploratory Assessments:

- 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
- Extramedullary hematopoiesis (EMH) mass size by MRI
- Spleen size by MRI
- Bone mineral density (BMD) by DXA
- 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 study although up to 64 patients may participate from the A536-04 study.

Analysis Populations:

The modified Intent-to-Treat (mITT) Population: All patients enrolled in the study who received at least one dose of ACE-536.

Efficacy Evaluable (EE) Population: All patients administered at least one dose of ACE-536 and have 1) at least three hemoglobin assessments over an 8-week period, not influenced by transfusion, in NTD patients, OR, 2) at least 8 consecutive weeks of transfusion frequency data in TD patients.

Pharmacokinetics Population: All patients who received at least 1 dose of ACE-536 during Study A536-06 and have sufficient serum ACE-536 values for PK analysis.

Primary Endpoint Analysis:

Safety analysis: To assess clinical safety, all AEs, vital sign measurements and clinical laboratory information will be summarized. AEs 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. Disposition rates will be summarized.

Efficacy/Pharmacodynamic Effects Analysis of Secondary Endpoints:

All efficacy analyses will be performed for the EE and mITT populations.

Efficacy will be evaluated by determining the proportion of β-thalassemia patients who have an erythroid response, defined as 1) a mean hemoglobin increase ≥ 1.0 g/dL or ≥ 1.5 g/dL over an 8-week or 12-week period as compared to baseline, not influenced by RBC transfusion in NTD patients or 2) ≥ 20% or ≥ 50% reduction in RBC transfusion burden over an 8-week or 12-week period as compared to the 8-week or 12-week period pretreatment in TD patients.

Baseline hemoglobin for NTD patients will be the average of two or more measurements performed during the screening period for base study A536-04 (for patients without treatment interruption) or study A536-06 (for patients with treatment interruption). Hemoglobin measurements within 2 weeks following RBC transfusion will be excluded.

Transfusion burden for TD patients will be defined as the ratio of RBC transfusion units (or mLs) transfused during an interval divided by the duration 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 8 or 12 weeks prior to Cycle 1 Day 1 of base study A536-04 (will also be analyzed using pretreatment period of study A536-06 for patients with treatment interruption). An interval during the treatment plus

follow-up period will be defined as an 8-week or 12-week interval after Cycle 1 Day 1 of study A536-06.

The erythroid response, as defined above, will also be evaluated using other pre-treatment and on-treatment intervals and other % reductions in RBC units transfused, including 100% (transfusion-free).

The erythroid response will be summarized using both a point estimate and its exact 95% confidence interval (CI) based on binomial distribution.

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

Analysis of other secondary efficacy/PD endpoints will include:

- Time to erythroid response and duration of erythroid response
- Change/percent change from pretreatment in mean transfusion burden in TD patients
- Change in mean hemoglobin level in NTD patients
- Change in pre-transfusion hemoglobin levels in TD patients (including analysis of patient subset with < 20% change in transfusion burden)
- Change from baseline in erythropoiesis parameters, including EPO, reticulocytes, nucleated RBCs, and soluble transferrin receptor
- Change from baseline in hemolysis parameters, including total/indirect bilirubin, and lactate dehydrogenase (LDH)
- Change from baseline in iron overload and iron metabolism parameters, including serum iron, TIBC, transferrin, calculated transferrin saturation, soluble transferrin receptor, ferritin, NTBI, hepcidin, iron chelation therapy use and LIC by MRI.

The exploratory endpoints will include evaluation of biomarkers related to the TGF-β superfamily, quality of life, change in EMH mass size, change in spleen size (in patient subset with no prior splenectomy), change in BMD, 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 CI 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% CI.

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 ADA on ACE-536 PK and drug exposure if ADA tests are deemed positive.

Pharmacokinetics analysis: PK parameters for ACE-536, such as maximum serum concentration (C_{max}), time to maximum concentration (t_{max}), and area under the concentration/time curve (AUC), will be estimated using non-compartmental and/or single compartment analysis, as appropriate. Dose proportionality may be assessed using the exposure data (e.g., C_{max}, AUC) 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, as appropriate.

2. SCHEDULE OF EVENTS

	Screening Day -28 to Day -1[1]	Cycle 1		Cycle 2	Cycle 3		Cycle 4	Cycle 5		Cycles 6-17: Repeat Cycles 2 through 5 three times[2,29]
		C1D1[1,2,13]	C1D8	C2D1[2,13]	C3D1[2,13]	C3D8	C4D1[2,13]	C5D1[2,13]	C5D8	
		Day 1	Day 8 (± 2d)	Day 22 (± 5d)	Day 43 (± 5d)	Day 50 (± 2d)	Day 64 (± 5d)	Day 85 (± 5d)	Day 92 (± 2d)	
Informed consent	X[1]									Cycles 6-17: Day 106 (± 2d) through 357 (± 2d)
Inclusion/Exclusion	X	X[2]								
Medical history[3]	X									
QoL Questionnaires[4]		X						X		
Physical examination[5]	X	X						(X)[5]		
Vital signs[6]	X	X		X	X		X	X		
ECG (12 lead)		X								
MRI for liver iron content[2, 7] (optional)		X						(X)[7]		
MRI for EMH masses[2, 8]		X						(X)[8]		
MRI for spleen size[2, 9]		X						(X)[9]		
DXA for BMD of total body, lumbar spine and total hip[29]		X						(X)[29]		
6MWT for NTD patients[30]		X						X		
Leg Ulcer Assessment[10]	Collected at each visit as applicable									
ECHO/MUGA (interruption patients only)	X[1]									
Serum iron studies[11]		X						X		
Serum folate[12]	X	X						(X)[12]		
Erythropoietin levels		X						X	X	
Hematology[13]	X	X	X	X	X	X	X	X	X	
Peripheral blood smear[14]		X						X		
Serum chemistry[15]	X	X			(X)[15]			X		
Urinalysis/urine chemistry[16]	X	X						X		
Anti-drug antibody[17]		X						X		
PK collection[18]		X	X	(X)[18]				(X)[18]	(X)[18]	

PD biomarkers[19]		X						X		
Hemoglobin Electrophoresis[20]		X						(X)[20]		
Pregnancy test[21]	X	X		X	X		X	X		
Menstrual History[22]	X	X		X	X		X	X		
Evaluate transfusion frequency/volume[23]	X	X		X	X		X	X		
Administer ACE-536		X		X	X		X	X		
Concomitant medications and AEs[24]	Collected Continuously									

	Cycle 18	Cycle 19	Cycle 20	Cycle 21	Cycle 22	Cycle 23	Cycles 24-87: Repeat Cycles 20 through 23 sixteen times[2,30]	EOT[25]	PTFU[26]	LTFU[27]	EOS[28]
	C18D1[2,13]	C19D1[2,13]	C20D1[2,13]	C21D1[2,13]	C22D1[2,13]	C23D1[2,13]		28 days (± 7 days) after the last dose of ACE-536 or at the time of discontinuation	2 months (± 7 days) after the last dose of ACE-536	Every 6 months (± 14 days) after the last dose of ACE-536	3 years after the last dose of ACE-536
	Day 358 (± 5d)	Day 379 (± 5d)	Day 400 (± 5d)	Day 421 (± 5d)	Day 442 (± 5d)	Day 463 (± 5d)					
QoL Questionnaires[4]				(X)[4]			Cycles 24-87: Day 484 (± 5d) through 1807 (± 5d)	X			
Physical examination[5]					(X)[5]			X			
Vital signs[6]	X	X	X	X	X	X		X			
MRI for liver iron content (optional)[7]				(X)[7]				X			
MRI for EMH masses[8]				(X)[8]				X			
MRI for spleen size[9]				(X)[9]				X			
DXA for BMD of total body, lumbar spine and total hip[31]				(X)[29]				X			
6MWT for NTD patients[32]				(X)[30]				X			
Leg Ulcer Assessment[10]	Collected at each visit as applicable							X			
Serum iron studies[11]				X				X	X		
Serum folate[12]				(X)[12]				X			
Erythropoietin levels				X				X	X		
Hematology[13]	X	X	X	X	X	X		X	X		
Peripheral blood smear[14]				X				X			
Serum chemistry[15]				X				X			
Urinalysis/urine chemistry[16]				X				X			
Anti-drug antibody[17]				X				X[17]			
PD biomarkers[19]				X				X			
Hemoglobin Electrophoresis[20]				(X)[20]				X			
Pregnancy test[21]	X	X	X	X	X	X		X			
Menstrual History[22]				X							
Evaluate transfusion frequency/volume[23]	X	X	X	X	X	X		X	X		
Administer ACE-536	X	X	X	X	X	X					
Concomitant medications and AEs[24]	Collected Continuously										
Malignancy and Pre-Malignancy Monitoring										X	X

[1] **Screening procedures:** Other than informed consent, procedures listed as part of the 28-day screening period are only applicable to patients with treatment interruption. ECHO or MUGA (only required for patients with treatment interruption) can be performed up to 56 days prior to C1D1. If performed as part of standard of care, ECHO/MUGA does not need to be repeated. Patients with treatment interruption will need to qualify per the additional inclusion criteria listed in Section 9.3.2. For patients without treatment interruption, C1D1 may coincide with EOT visit of the base study A536-04. Procedures that are required to confirm eligibility for patients without treatment interruption can be performed at the base study A536-04 EOT visit and used to confirm eligibility prior to dosing on C1D1 of study A536-06.

[2] **Study procedures** must be done prior to administration of study drug. Note that all windows on visits should be determined relative to the date of the previous dose of ACE-536. For patients without treatment interruption, C1D1 procedures shaded grey may be conducted as part of the EOT visit for study A536-04 and may not need to be repeated for study A536-06. All patients must be assessed for eligibility prior to dosing on C1D1. C1D1 MRI for LIC (at selected sites) and EMH masses (unless site does not have feasibility to perform the assessment) and MRI of the spleen (if no prior splenectomy) can be performed up to 56 days prior to C1D1. If performed as part of standard of care, MRI for LIC, MRI for EMH and spleen size do not need to be repeated. All screening and C1D1 procedure results required to confirm eligibility must be obtained and reviewed prior to study drug administration in A536-06. **On dosing days:** Note that the patient dose must be calculated based on the patient's weight on the day of dosing. Starting dose level for C1D1, dose modification rules and titration rules must be reviewed and implemented prior to dosing as required per protocol (see Section 10.7, Section 10.8 and Section 10.9). If a dose delay is required per the dose modification rules (Section 10.8), the patient will not be dosed. The patient will return weekly for assessment of hematology results and AEs until the patient is eligible to receive the next dose of ACE-536 and start the next cycle. The patient should resume the study at the planned dosing cycle (e.g. if the patient missed a dose at C4D1, then they would resume dosing at C4D1 and not skip to C5D1).

[3] **Medical history:** Medical history for patients with treatment interruption will include medical events occurring after EOS visit in study A536-04 and prior to C1D1 for study A536-06. Medical history for patients without treatment interruption will be taken from study A536-04.

[4] **Quality of life questionnaires** are required at C1D1, C5D1, C9D1, C13D1 C17D1, C25D1, C33D1, C41D1, C49D1, C57D1, C65D1, C73D1, C81D1 and EOT.

[5] **Physical exam:** Physical exam should also include an optional evaluation of gonadal size of male patients on C1D1, C5D1, C13D1, C22D1, C30D1, C38D1, C46D1, C54D1, C62D1, C70D1, C78D1 EOT and EOS.

[6] **Vital signs** will include weight, heart rate, and SBP and DBP. 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.

[7] **MRI for liver iron content** will be performed at selected sites at C1D1, C9D1 C17D1, C25D1, C33D1, C41D1, C49D1, C57D1, C65D1, C73D1, C81D1 and EOT within a +/- 10-day window for each scan. A MRI for liver iron content is not required if < 3 months since the previous MRI for liver iron content, unless clinically indicated.

[8] **MRI of the chest and abdomen for EMH mass measurement** will be performed for at selected sites at C1D1. If the assessment at C1D1 is negative (no masses), additional scans are not required unless clinically indicated. If the assessment at C1D1 is positive (masses present), repeat MRI at C9D1, C17D1, C25D1, C33D1, C49D1, C65D1, C81D1 and EOT within a +/- 10-day window for each scan. A MRI of the chest and abdomen for EMH mass measurement is not required if < 3 months since the previous MRI of the chest and abdomen for EMH mass measurement, unless clinically indicated

[9] **MRI of the spleen:** will be performed at selected sites at C1D1 on patients with no prior splenectomy. If there is no indication of an enlarged spleen on the C1D1 scan, additional scans are not required unless clinically indicated. If there is an enlarged spleen on the C1D1 scan, repeat imaging at C9D1 C17D1, C25D1 C33D1, C49D1, C65D1, C81D1 and EOT within a +/- 10-day window for each scan. Patients with clinical signs of a change in spleen size or abnormality should have an abdominal ultrasound or MRI performed as needed throughout the study. A MRI for the spleen is not required if < 3 months since the previous MRI for the spleen, unless clinically indicated.

[10] **Leg ulcer assessment:** Patients with leg ulcers should have regular assessment of the leg ulcer(s) throughout the study. Photographs of the leg ulcer(s) pre- and post- dose should be provided when available to document any changes in the ulcer(s).

[11] **Serum iron studies:** May include serum iron, TIBC, transferrin, soluble transferrin receptor, calculated transferrin saturation, ferritin, NTBI. The sponsor may perform additional sample analysis for biomarkers for exploratory research purposes only.

[12] **Serum folate:** Required at screening for patients with treatment interruption to confirm eligibility. Required for all patients at C1D1, C5D1, C13D1, C21D1, C29D1 C37D1, C45D1, C53D1, C61D1, C69D1, C77D1, and EOT.

[13] **Hematology:** RBC, nucleated red blood cells (nRBC) (local and central lab), white blood cell (WBC) with differential, hemoglobin, hematocrit, reticulocyte count, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red blood cell distribution width (RDW). On dosing days, hemoglobin values are to be drawn and resulted (up to 24 hours) prior to dosing (see Section 10.8, Patient Dose Modification Rules).

[14] **Peripheral blood smear:** Peripheral blood smears will be prepared centrally from blood sample.

[15] **Chemistry**: Sodium, potassium, AST, ALT, 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. Perform at screening, C1D1, C3D1, C5D1, C9D1, C13D1, C17D1, C21D1, C25D1, C29D1, C33D1, C37D1, C41D1, C45D1, C49D1, C53D1, C57D1, C61D1, C65D1, C69D1, C73D1, C77D1, C81D1, EOT and EOS.

[16] **Urinalysis by dipstick analysis (local lab):** pH, specific gravity, protein, glucose, ketones, blood, leukocyte esterase, and nitrite, with microscopic examination if indicated. **Urine chemistry (central lab):** urine chemistries include but are not limited to: microalbumin and creatinine.

[17] 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.

[18] **PK collection** is only required on C1D1, C1D8, C2D1, C9D1, C9D8, C10D1, C17D1, and C17D8.

[19] **PD biomarkers:** May include hepcidin, GDF15, GDF8, GDF11, activin A and others to be determined. The sponsor may perform additional sample analysis for biomarkers for exploratory research purposes only.

[20] **Hemoglobin electrophoresis:** Samples will be collected for central hemoglobin electrophoresis on C1D1, C5D1, C13D1, C21D1, C29D1, C37D1, C45D1, C53D1, C61D1, C69D1, C77D1, EOT, and EOS.

[21] **Pregnancy test:** (urine or serum) is required for female patients of child bearing potential only.

[22] **Menstrual history:** is required for female patients of child bearing potential only

[23] **Transfusion frequency/volume:** Transfusion history will be collected from the EOS visit in study A536-04 through the C1D1 visit of A536-06 as available; TD patients will have a "pre-transfusion hemoglobin threshold" for requiring transfusion during the study which will be determined based on transfusion history. For TD patients without treatment interruption, baseline pre-transfusion hemoglobin threshold will be the mean of all documented pre-transfusion hemoglobin values during the 12 weeks prior to C1D1 of base study A536-04 or study A536-06 (for TD patients with treatment interruption). During treatment, if the pre-transfusion hemoglobin level 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).

[24] **Adverse events:** Patients should be monitored for AEs throughout the study. 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.

[25] **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 EOT visit at the time of discontinuation.

[26] **Post-Treatment Follow-Up (PTFU):** Should be performed 2 months (± 7 days) after the last dose of ACE-536.

[27] **Long-Term Follow-Up (LTFU)**: Should be performed every 6 months (± 14 days) after the last dose of ACE-536 for 3 years after the last dose of ACE-536 to monitor for presence of malignancy and pre-malignancy, as per standard of care.

[28] **End of Study (EOS):** Should be performed 3 years after the last dose of ACE-536.

[29] **Cycles 6 through 17:** Procedures and visits for Cycles 6 through 9, 10 through 13, and 14 through 17 follow the same procedures and visits as scheduled for Cycles 2 through 5, with some exceptions as noted above.

[30] **Cycles 24 through 87:** Patients may not exceed 87 cycles or 1825 days after first dose, whichever occurs first. The last dose of ACE-536 may not be administered after Day 1825, regardless of the number of cycles completed. Procedures and visits for Cycles 24 through 27, 28 through 31, 32 through 35, 36 through 39, 40 through 43, 44 through 47, 48 through 51, 52 through 55, 56 through 59, 60 through 63, 64 through 67, 68 through 71, 72 through 75, 76 through 79, 80 through 83 and 84 through 87 follow the same procedures and visits as scheduled for Cycles 20 through 23, with some exceptions as noted above.

[31] **DXA for BMD** will be performed at selected sites at C1D1, C9D1, C17D1, C25D1, C33D1, C49D1, C65D1, C81D1 and EOT within a +/- 10-day window for each scan. A DXA for BMD is not required if < 3 months since the previous DXA for BMD, unless clinically indicated.

[32] **6MWT for NTD patients** will be performed at selected sites at C1D1, C5D1, C9D1, C13D1, C17D1, C25D1, C33D1, C41D1, C49D1, C57D1, C65D1, C73D1, C81D1 and EOT.

3. TABLE OF CONTENTS

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
BP	Blood pressure
BUN	Blood urea nitrogen
CXDY	Cycle X Day Y
CI	Confidence interval
C_{max}	Maximum concentration
CREAT	Creatinine
CRF	Case report form
CRO	Contract research organization
DBP	Disastolic blood pressure
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
EPO	Erythropoietin
EOT	End of treatment
FACT-An	Functional Assessment of Cancer Therapy-Anemia Scale
FDA	United States Food and Drug Administration

Abbreviation	Definition
FSH	Follicle stimulating hormone
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
LIC	Liver iron content
LDH	Lactate dehydrogenase
LMW	Low molecular weight
LTFU	Long-term follow-up
MedDRA	Medical Dictionary for Regulatory Activities
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
miTT	Modified intent-to-treat
MRI	Magnetic resonance imaging
MUGA	Multi gated acquisition scan
NCI-CTCAE	National Cancer Institute-Common terminology Criteria for Adverse Events
nRBCs	Nucleated red blood cells
NTBI	Non-transferrin bound iron
NTD	Non-transfusion dependent
PD	Pharmacodynamic

Abbreviation	Definition
PHI	Protected health information
PK	Pharmacokinetic
PTFU	Post-treatment follow-up
QoL	Quality of life
RBC	Red blood cell
RDW	Red blood cell distribution width
SAE	Serious adverse event
SAP	Statistical analysis plan
SBP	Systolic blood pressure
SC	Subcutaneous
SD	Standard deviation
SRT	Safety review team
SF-36	Short Form (36) Health Survey
SUSAR	Suspected unexpected serious adverse reaction
SVT	Superficial venous thrombosis
TD	Transfusion dependent
$T_{1/2}$	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 Independent Ethics Committee (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 patient. 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, (erythropoiesis). In nonclinical experiments, ACE-536 has been shown to bind with high affinity to some TGF-β ligands (e.g., GDF8, GDF11, BMP6 and activin B) but substantially less, or not at all, to others (e.g., BMP10 and activin A). The emerging body of evidence on ACE-536 suggests that its mechanism of action is completely independent from that of erythropoietin (EPO), and involves stimulation of the later, maturation phase of erythroblast differentiation and maturation in the bone marrow.

7.1. Overview of Target Indication

The target indication for ACE-536 is for the treatment of 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 02 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.[6] Children with β-thalassemia who are periodically transfused to maintain a hemoglobin value of 9.5 to 14 g/dL grow and develop normally. 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 nonclinical pharmacology studies have been conducted with ACE-536 or its murine ortholog, RAP-536. The RAP-536 molecule has the same ECD as ACE-536, but contains a murine IgG Fc domain in place of the human IgG Fc. The RAP-536 ortholog is substantially less immunogenic in rodent species and therefore permitted the conduct of longer term pharmacology studies in rodents without the confounding influence of immune reactivity.

ACE-536 has been shown to have significant effects on erythropoiesis. Evidence suggests that it binds ligands that normally act to modulate the differentiation of late-stage erythroid precursors (normoblasts). Inhibition of these ligands leads to a rapid and robust erythroid response and increased production of mature erythrocytes. Because ACE-536 affects late-stage erythrocyte precursor populations, its action is distinct from that of EPO. Unlike EPO, ACE-536 is not a growth-promoting cytokine.

Studies of ACE-536 in normal animals across several species (mice, rats, and cynomolgus monkeys) demonstrated an erythroid response that was rapid in onset, robust, and sustained. These effects were also demonstrated in a variety of animal models of anemia, including anemia associated with kidney failure, acute blood loss, and chemotherapy.

RAP-536 was shown to significantly improve hematologic parameters, including increased hemoglobin concentrations, and to correct ineffective erythropoiesis and anemia in mouse models of myelodysplastic syndromes (MDS) and β-thalassemia, both target indications for ACE-536. This is consistent with its proposed mechanism of action of driving the late stages of erythroid differentiation that is downstream and distinct from EPO.

The Hbb $^{major-/-}$ transgenic mouse was used to test RAP-536 in a model of β-thalassemia. This model represents human β–thalassemia intermedia, which is characterized by severe anemia, failure of erythroid differentiation, increased apoptosis of erythroid precursors, erythroid hyperplasia, splenomegaly and iron overload. Treatment with RAP-536 led to significant improvement in hematological parameters, including RBC and hemoglobin. Further effects of

treatment included decreased reticulocytes, red cell distribution width, EPO, bilirubin, RBC inclusion bodies, reactive oxygen species, serum ferritin, liver iron content (LIC), and spleen size, as well as increased hepcidin and bone mineral density, and improvements in RBC morphology.

Additional information regarding pharmacological effects of ACE-536 is summarized in the current version of the ACE-536 IB.

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. In addition, the main phase of a repeat-dose toxicology study of 6 months treatment duration in cynomolgus monkeys is complete. Findings from toxicology studies are described in more detail in the IB.

7.3. Summary of Clinical Experience

The safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) 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 PK parameters and PD effects of ACE-536.

A total of 32 subjects were enrolled. Eligible subjects were healthy, postmenopausal women, 45-75 years old. The anticipated PD effect was an increase in plasma hemoglobin level. Screening and baseline hemoglobin values had to be between 11.0 and 14.5 g/dL, inclusive, to mitigate the risk of substantial excursions above the normal range. Sequential cohorts of 8 subjects each were randomized to receive either ACE-536 (n=6) or placebo (n=2) administered as a SC injection on Day 1 and Day 15. Each subject was to be followed for 112 days (16 weeks) after receiving their last dose of ACE-536 or placebo. The following dose levels were evaluated: 0.0625, 0.125, and 0.25 mg/kg.

ACE-536 administered every 2 weeks by subcutaneous (SC) injection for one or two doses at dose levels of 0.0625, 0.125 or 0.25 mg/kg was generally safe and well-tolerated in healthy postmenopausal female subjects. No serious or severe adverse events (AE) were reported. The majority of AEs were considered mild in severity. AEs that were considered at least possibly drug-related across ACE-536 dose groups included injection site hemorrhage (3 subjects), injection site macule (2 subjects), and dry skin, macule, hyperesthesia, muscle spasms, myalgia, generalized pruritus, and papular rash (1 subject each). Injection site pain was reported in one placebo-treated subject.

PK results indicated mean AUC and C_{max} values increased in a dose-proportional manner with ACE-536 dose level; mean T_{max} ranged from 7.0 to 9.8 days, and mean T½ ranged from 14.9 to 16.2 days after the first dose. These PK results support intermittent SC dosing of ACE-536 in future studies, e.g., every 3 weeks.

PD assessments for hematological markers showed increases in hemoglobin levels at the 0.125 mg/kg dose level and more consistently at the 0.25 mg/kg dose level. Small increases in

reticulocyte count and EPO levels were also observed at the two higher dose levels of ACE-536 investigated. A small decrease in serum follicle stimulating hormone (FSH) levels was observed at 0.25 mg/kg. These data support further studies of ACE-536 in conditions associated with ineffective erythropoiesis and anemia.

Two phase 2 studies with ACE-536 are ongoing. Study A536-03 is entitled, "A Phase 2, Open-Label, Ascending Dose Study of ACE-536 for the Treatment of Anemia in Patients with Low or Intermediate-1 Risk Myelodysplastic Syndromes (MDS)". Study A536-04 is entitled "A Phase 2, Open-Label, Ascending Dose Study to Evaluate the Effects of ACE-536 in Patients with ß-Thalassemia". Preliminary results from these studies and additional information regarding the completed Phase 1 study can be found in the current version of the IB.

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 patients and dose escalation/reduction rules will be utilized to minimize risks associated with increased RBC parameters.

AEs observed in the Phase 1 study in healthy volunteers and the ongoing phase 2 studies that were considered probably or possibly related to study drug included injection site reactions (hemorrhage, pruritus, rash), skin rash, hyperesthesia, muscle spasms, myalgia, pruritus, and hyperkalemia.

As with all biologics, there is the potential for anti-drug antibodies (ADA) that can be associated with increased drug clearance and hypersensitivity reactions. ADA formation against ACE-536 as well as human ActRIIB protein will be monitored in the initial clinical 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. In addition, the main phase of a repeat-dose toxicology study of 6 months treatment duration in cynomolgus monkeys is complete. Reproductive toxicity studies are currently ongoing with ACE-536 and therefore ACE-536 should not be administered to pregnant or nursing 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 treatment and for at least 12 weeks following the last dose of ACE-536. Please refer to the Investigator's Brochure for additional information regarding findings from toxicology studies. 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 AE 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 IB. The ACE-536 IB should be reviewed prior to initiating the study.

8. TRIAL OBJECTIVES

8.1. Primary Objectives

- To evaluate the long-term safety and tolerability of ACE-536 in patients with β-thalassemia who were previously enrolled in study A536-04.

8.2. Secondary Objectives

- To evaluate erythroid response defined as the proportion of patients with:
    - Mean hemoglobin increase ≥ 1.5 g/dL over a continuous 12-week interval compared to baseline in non-transfusion dependent (NTD) patients, not influenced by RBC transfusion, OR
    - Reduction in RBC transfusion burden by ≥ 50% over a continuous 12-week interval compared to the 12 weeks prior to the start of treatment in transfusion dependent (TD) patients.
- To evaluate erythroid response defined as proportion of patients with:
    - Mean hemoglobin increase ≥ 1.5 g/dL over a continuous 8-week interval compared to baseline in NTD patients, not influenced by RBC transfusion, OR
    - Reduction in RBC transfusion burden by ≥ 20% over a continuous 8-week interval compared to the 8 weeks prior to the start of treatment in TD patients.
- To evaluate the time to erythroid response and duration of erythroid response.
- To evaluate the mean change from baseline over an 8- or 12-week period in hemoglobin level in NTD patients not influenced by RBC transfusion
- To evaluate the mean % change from baseline in transfusion burden over an 8- or 12-week period in TD patients.
- To evaluate the mean change in pre-transfusion hemoglobin levels in TD patients
- To evaluate changes in markers of erythropoiesis, hemolysis, iron overload, and iron metabolism
- To examine the PK profile of ACE-536 in patients with β-thalassemia

8.3. Exploratory Objectives

- To evaluate biomarkers related to the TGF-β superfamily
- To evaluate patient self-reported quality of life using tools including but not limited to the Functional Assessment of Cancer Therapy-Anemia Scale (FACT-An) and Short Form (36) Health Survey (SF-36) questionnaires
- To evaluate change in extramedullary hematopoiesis (EMH) mass size by MRI
- To evaluate change in spleen size by MRI
- To evaluate change in bone mineral density (BMD) by DXA

- To evaluate change in leg ulcers
- To evaluate change in the 6-minute walk test (6MWT) distance in NTD patients

9. OVERALL STUDY DESIGN AND PLAN: DESCRIPTION

This open-label extension study will evaluate the effects of up to 60 months of ACE-536 treatment in patients with β-thalassemia previously enrolled and treated with ACE-536 for up to 3 months in study A536-04.

9.1. Study Design

The base study (A536-04) is a phase 2, open-label, ascending dose study to evaluate the effects of ACE-536 in patients with β-thalassemia. A total of up to 64 patients may be enrolled in study A536-04 and may be eligible for study A536-06.

Consenting patients that meet the A536-06 eligibility criteria may immediately roll over from A536-04 to study A536-06 following the last ACE-536 dose. These patients may forego the End of Study (EOS) visit in study A536-04 to begin study A536-06. For these patients, Cycle 1 Day 1 (C1D1) of study A536-06 may take place 28 (± 7) days after the last dose administered in study A536-04, which may coincide with the patient's A536-04 End of Treatment (EOT) visit. These patients are considered "patients without treatment interruption".

Patients who complete the EOS visit for study A536-04 and are ≥ 28 days post EOS visit are considered "patients with treatment interruption" and will be re-assessed for eligibility by meeting all eligibility criteria plus additional inclusion criteria, as defined in Section 9.3.

Patients who do not meet the above criteria (e.g., > 35 days after last dose in study A536-04 and < 28 days post EOS visit in study A536-04) may still participate, but should be treated as patients with treatment interruption and should not begin study A536-06 C1D1 until they have reached ≥ 28 days post EOS visit from study A536-04 so that new baseline assessments can be measured.

For patients without treatment interruption, transfusion status (NTD or TD) will carry-over from the base study A536-04. For NTD patients with treatment interruption, transfusion status will be reassessed prior to C1D1 of study A536-06. NTD patients with treatment interruption are defined as patients who require transfusion of < 4 units of RBCs over the 8 weeks prior to Cycle 1 Day 1 of study A536-06. For TD patients with treatment interruption, transfusion status will carry-over from the base study A536-04.

A patient without treatment interruption may continue to be dosed with ACE-536 at the same dose level (rounded to nearest A536-06 starting dose level) administered at their last dose in study A536-04 (unless a dose reduction is required based upon patient dose modification rules from study A536-06). All patients with treatment interruption will be initially treated with ACE-536 at a starting dose level of 0.8 mg/kg which has been determined to be safe and well tolerated by the Safety Review Team (SRT) based on data from study A536-04.

Each TD patient 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 of base study

A536-04 (for patients without treatment interruption) or study A536-06 (for patients with treatment interruption). During treatment, if the pre-transfusion hemoglobin level 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).

Patients will participate in the extension study A536-06 for up to 97 months, including a 28-day (1 month) screening period, a 60-month treatment period and a 3-year (36 months) follow-up period.

The SRT established for study A536-04 will review safety data and PD data periodically throughout study A536-06.

9.2. Discussion of Study Design

The primary endpoint is evaluating the long-term safety and tolerability of ACE-536 administration in patients with β-thalassemia who completed participation in A536-04. To support this endpoint, this study includes safety measurements that are considered standard for studies with investigational medications.

An open-label study, conducted in a limited number of patients, with protocol-specified procedures for dose reduction is considered appropriate for a long-term safety study in this target patient population. Individualization of dose level, 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.

Secondary endpoints were selected to evaluate the impact of ACE-536 on efficacy and PD parameters, including effects on hematologic parameters and on complications of thalassemia.

The initial dose level of 0.8 mg/kg has been selected for patients with treatment interruption based upon data from the ongoing A536-04 study. Dose levels utilized in this study will not exceed maximum dose levels tested in study A536-04.

9.3. Selection of Study Population

9.3.1. Transfusion Status

- For patients without treatment interruption patient transfusion status (NTD or TD) will carry-over from the base study A536-04.
- For NTD patients with treatment interruption, patient transfusion status will be reassessed prior to C1D1 of study A536-06.
  - NTD patients with treatment interruption are defined as patients who require transfusion of < 4 units of RBCs over the 8 weeks prior to Cycle 1 Day 1 of study A536-06.
- For TD patients with treatment interruption, patient transfusion status will carry-over from the base study A536-04.

9.3.2. Inclusion Criteria

All patients must meet the following criteria:

1. Completion of the treatment period in the base study A536-04.
2. 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.
3. Patient is able to adhere to the study visit schedule, understand and comply with all protocol requirements.
4. Patient understands and is able to provide written informed consent.

All patients with treatment interruption must also meet the following criteria:

Patients with treatment interruption (defined as patients who complete the EOS visit for study A536-04 and are ≥ 28 days post EOS visit) must also meet the following criteria:

5. Mean hemoglobin concentration < 10.0 g/dL of 2 measurements (not influenced by RBC transfusion) (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 NTD patients.
6. Adequate folate levels or on folate therapy.
7. Platelet count ≥ 100 x 10^9/L and ≤ 1,000 x 10^9/L.
8. Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) < 3 x upper limit of normal (ULN).
9. Serum creatinine ≤ 1.5 x ULN.
10. Ejection fraction ≥ 50% by Echocardiogram (ECHO) or Multi gated acquisition scan (MUGA).

9.3.3. Exclusion Criteria

All patients must not meet any of the following criteria:

1. Discontinuation/withdrawal from study A536-04 due to patient request, patient unwillingness or inability to comply with the protocol, pregnancy, use of prohibited medication (e.g., hydroxyurea), medical reason or AE, hypersensitivity reaction to the study drug, at the discretion of the sponsor, or loss to follow-up prior to completion of the treatment period.
2. 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.

3. Symptomatic splenomegaly.
4. Splenectomy within 56 days prior to Cycle 1 Day 1.
5. 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.
6. Patients receiving or planning to receive hydroxyurea treatment. Patients must not have had hydroxyurea within 90 days of Cycle 1 Day 1.
7. For patients with treatment interruption: Iron chelation therapy if initiated within 56 days prior to Cycle 1 Day 1.
8. 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 and low molecular weight (LMW) heparin for superficial venous thrombosis (SVT) is permitted).
9. Treatment with another investigational drug (including sotatercept [ACE-011]) 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 at any time between the end of treatment of the base study A536-04 and Cycle 1 Day 1.
10. Known positive for human immunodeficiency virus (HIV), active infectious hepatitis B (HBV) or active infectious hepatitis C (HCV).
11. Uncontrolled hypertension defined as systolic blood pressure (SBP) ≥ 150 mm Hg or diastolic blood pressure (DBP) ≥ 100 mm Hg.
12. 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).
13. Pregnant or lactating females.
14. History of severe allergic or anaphylactic reactions or hypersensitivity to recombinant proteins or excipients in the investigational drug.
15. Any other condition not specifically noted above which, in the judgment of the investigator, would preclude the patient from participating in the study.

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 AE, 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 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 an SC injection on Cycle 1 Day 1. Subsequent doses will be administered every 3 weeks on Day 1 of the cycle for up to 87 cycles. Patients may not exceed 87 cycles or 1825 days after first dose, whichever occurs first. The last dose of ACE-536 may not be administered after Day 1825, regardless of the number of cycles completed. Dose reductions may be required for individual patients as outlined in the Patient Dose Modifications Rules (Section 10.8). If a dose delay is required, the patient will return weekly for assessment of hematology results and AEs 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. Dose level titration increases may also occur for individual patients as outlined in the Individual Dose Titration rules (Section 10.9). If a patient has a positive 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). 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. For patients without treatment interruption, any concomitant medication ongoing at the time of EOT in A536-04 needs to be re-entered as a concomitant medication in A536-06. For patients with treatment interruption, 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. Prohibited Concomitant Medications and Procedures

No concurrent treatments with any other investigational agent are allowed.

The following are not allowed during the treatment period: hematopoietic growth factors, anticoagulant therapies for deep vein thrombosis (DVT), and platelet aggregation inhibitors (with the exception of aspirin and LMW heparin). If a patient requires use of these therapies, the patient should be discontinued from the study treatment.

10.2.2. 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 EOT visit and enter the follow-up period. Iron chelation therapy is allowed if initiated at least 56 days prior to C1D1. If iron chelation therapy is required during study treatment, patient should follow standard of care for management of iron chelation therapy.

10.2.3. 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 comorbidity.

For any RBC transfusions (NTD or TD patients) received during the study, collect hemoglobin values just prior to transfusion.

Each TD patient 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 of base study A536-04 (for patients without treatment interruption) or study A536-06 (for patients with treatment interruption). During treatment, if the pre-transfusion hemoglobin level 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). Patients may be transfused on the same day as ACE-536 is administered except on C1D1 for patients with treatment interruptions.

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 study does not require randomization and is open-label.

10.5. Treatments Administered

A patient without treatment interruption may continue to be dosed with ACE-536 at the same dose level (rounded to nearest A536-06 starting dose level) administered at their last dose in study A536-04 (unless a dose reduction is required based upon patient dose modification rules from study A536-06). All patients with treatment interruption will be initially treated with ACE-536 at a starting dose level of 0.8 mg/kg which has been determined to be safe and well tolerated by the SRT based on data from study A536-04. Please refer to Table 2, Examples of Possible Starting Dose with Dose Modifications (Reductions and Titrations), for additional information regarding treatments administered.

10.6. Safety Evaluation

Safety will be evaluated by the 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 periodically throughout the trial, including AEs, SAEs, laboratory results (including hematology and chemistry), vital signs, and erythroid response data.

10.7. Starting Dose Levels

A patient without treatment interruption may continue to be dosed with ACE-536 at the same dose level (rounded to nearest A536-06 starting dose level) administered at their last dose in study A536-04 (unless a dose reduction is required based upon patient dose modification rules from study A536-06). All patients with treatment interruption will be initially treated with ACE-536 at a starting dose level of 0.8 mg/kg which has been determined to be safe and well tolerated by the Safety Review Team (SRT) based on data from study A536-04. Examples of possible starting dose levels with dose modifications (reductions and titrations) are below for reference.

Table 2: Examples of Possible Starting Dose with Dose Modifications (Reductions and Titrations)

3rd Dose Reduction	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
0.2 mg/kg	0.4 mg/kg	0.6 mg/kg	0.8 mg/kg	1.0 mg/kg	1.25 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		

- Starting dose level for TD and NTD patients to be determined based on data from study A536-04 (range: 0.2-1.25 mg/kg).
- Starting dose level for patients without treatment interruption will be last dose level (rounded to nearest A536-06 starting dose level) administered in study A536-04 (unless a dose reduction is required based upon patient dose modification rules from study A536-06).
- Patients may be titrated up and down dose levels as required per protocol to meet dose modification and titration rules.
- Patients who require more than 2 dose reductions due to an AE should be discontinued from treatment and complete the EOT and EOS visits.
- The maximum dose titration will not exceed the maximum dose level tested in base study A536-04.

10.8. Individual Dose Modification Rules

The following dose modification rules include both PD and safety parameters which may require a dose delay and/or a dose reduction. These rules should be assessed prior to every dosing visit for all patients. If a dose delay is required, the patient should have weekly visits to assess hematology results and AEs until the patient is eligible to receive the next dose of ACE-536.

Observation on Dosing Day	Action	ACE-536 Dose Modification
Hemoglobin ≥ 13 g/dL	Hold dose and monitor patient weekly until hemoglobin < 12 g/dL	Resume dosing once hemoglobin < 12 g/dL at the same dose level
Hemoglobin increase ≥ 2 g/dL from the previous dosing day (not attributable to RBC transfusion)	Continue dosing	Reduce the dose by 1 dose level
Non-hematologic AE ≥ Grade 3, or hematologic AE ≥ 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 the dose by 1 dose level[a]
Grade 3 leukocytosis (WBC >100,000/μL)		Discontinue treatment

[a] Patients who require more than 2 dose reductions due to an AE should be discontinued from treatment and complete the EOT, PTFU, LTFU, and EOS visits.

10.9. Individual Dose Titration

10.9.1. Individual Dose Titration for NTD Patients

For all NTD patients (with and without treatment interruption), the dose level will be assessed for titration at Day 1 of every cycle starting at C3D1 (e.g., C3D1, C4D1, C5D1, etc.). The dose level should be titrated individually for each patient not to exceed the maximum dose level evaluated in the base study A536-04 as follows:

- If hemoglobin increase from baseline is < 1.5 g/dL throughout the previous two 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 two consecutive measurements during the previous two 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 two consecutive measurements during the previous two 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 two or more measurements performed during the screening period for base study A536-04 (for patients without treatment interruption) or study A536-06 (for patients with treatment interruption). Hemoglobin measurements within 2 weeks following RBC transfusion will be excluded from both the calculation of the mean baseline hemoglobin and for evaluation of dose titration for a patient during treatment.

10.9.2. Individual Dose Titration for TD Patients

For all TD patients (with and without treatment interruption), the dose level will be assessed for titration at Day 1 of every cycle starting at C3D1 (e.g., C3D1, C4D1, C5D1, etc.). The dose

level should be titrated individually for each patient not to exceed the maximum dose level evaluated in the base study A536-04 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).

10.10. 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 or interruption, the investigator should consult with the medical monitor to decide whether to continue dosing at the same dose level, reduce the dose level, delay the patient's dose, or discontinue the patient's treatment with ACE-536.

Patient treatment interruption for site administrative reasons will be allowed between Cycle 17 and 18 and between Cycle 35 and 36 upon discussion with the sponsor.

11. STUDY PROCEDURES

Please refer to Section 2, Schedule of Events 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

11.2.1. Local Laboratory Testing

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, nucleated red blood cells (nRBCs), white blood cell (WBC) with differential, hemoglobin, hematocrit, reticulocyte count, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red blood cell distribution width (RDW).
- Serum chemistry: Sodium, potassium, 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.
- Serum folate: required at screening for patients with treatment interruption to confirm eligibility; required at C1D1 and as required per schedule of events for all patients

11.2.2. Central Laboratory Testing

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.

- nRBCs
- EPO levels
- Peripheral blood smear
- Serum iron studies
- Urine chemistries include but are not limited to: microalbumin and creatinine

11.3. Other Safety Assessments

- Physical examination (including optional evaluation of gonadal size in males at specified timepoints)
- Vital signs: weight, heart rate, systolic and diastolic blood pressure.
- 12-lead ECG
- ADA testing: (Blood samples should be drawn and processed on-site for serum collection at the time points specified according to the relevant Laboratory Manual)
- The occurrence of a new malignancy or premalignant lesion will be monitored as an event of interest and should be included as part of the assessment of adverse events throughout the course of the study. Investigators are to report the development of any new malignancy or premalignant lesion as a serious adverse event, regardless of causal relationship to study drug, occurring at any time for the duration of the study, from the time of signing the ICF up to and including 3 years of follow up.

11.4. Pharmacokinetic and Pharmacodynamic Assessments

11.4.1. Pharmacokinetic Assessments

PK assessment of ACE-536 concentrations will be performed periodically as outlined in Section 2, Schedule of Events. 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 relevant Laboratory Manual.

11.4.2. Pharmacodynamic Evaluations

PD assessments including hematologic laboratory assessments and transfusion information will be used to determine erythroid response to ACE-536. Please note specific timepoints according to the Schedule of Events (Section 2):

- An MRI will be performed at selected sites to evaluate LIC.
- An MRI will be performed to evaluate EMH masses (unless site does not have the feasibility to perform the assessment).
- A DXA will be performed at selected sites to evaluate total body, lumbar spine and total hip BMD.
- Patients with leg ulcers should have regular assessment of the leg ulcer(s) throughout the study. Photographs of the leg ulcer(s) pre- and post- dose should be provided when available to document any changes in the ulcer.
- A 6MWT will be performed at selected sites for NTD patients.
- Quality of Life tools including by not limited to the FACT-An and SF-36 questionnaires will be assessed.
- Biomarkers for iron metabolism may include serum iron, TIBC, transferrin, soluble transferrin receptor, calculated transferrin saturation, ferritin, and NTBI.

- Biomarkers related to the TGFβ superfamily and/or iron metabolism, such as hepcidin, GDF15, GDF8, GDF11, Activin A and others may be tested in blood samples, to be determined.
- Hemoglobin electrophoresis samples will be collected for central analysis.
- The sponsor may perform additional sample analysis for biomarkers for exploratory research purposes only.

12. STUDY SCHEDULE

Please refer to Section 2, Schedule of Events 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 during the 28-day screening period for all patients.
- Other than informed consent, procedures listed as part of the 28-day screening period are only applicable to patients with treatment interruption.
- ECHO or MUGA (only required for patients with treatment interruption) can be performed up to 56 days prior to C1D1. If performed as part of standard of care, ECHO/MUGA does not need to be repeated.
- Patients with treatment interruption (defined as patients who complete the EOS visit for study A536-04 and are ≥ 28 days post EOS visit) will need to qualify per the additional inclusion criteria listed in Section 9.3.2.
- Transfusion history will be collected from the EOS visit in study A536-04 through the C1D1 visit of A536-06 as available; TD patients 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. For TD patients without treatment interruption, the baseline pre-transfusion hemoglobin threshold will be the mean of all documented pre-transfusion hemoglobin values during the 12 weeks prior to C1D1 of base study A536-04 or study A536-06 (for TD patients with treatment interruption).
- Pregnancy test (urine or serum) and menstrual history is required of female patients of child bearing potential only.
- Medical history for patients with treatment interruption will include medical events occurring after EOS visit in study A536-04 and prior to C1D1 for study A536-06. Medical history for patients without treatment interruption will be taken from study A536-04.
- 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.
- Screen failure information will be maintained to document specific information, including but not limited to, reason for failure.

12.2. Dosing Days and Interim Visits

- Study procedures must be done prior to administration of study drug. All patients must be assessed for eligibility prior to dosing on C1D1. C1D1 MRI for LIC

(at selected sites) and EMH masses (unless site does not have feasibility to perform the assessment) and MRI of the spleen (if no prior splenectomy) can be performed up to 56 days prior to C1D1. If performed as part of standard of care, MRI for LIC, MRI for EMH and spleen do not need to be repeated. All screening and Cycle 1 Day 1 procedure results required to confirm eligibility must be obtained and reviewed prior to study drug administration in A536-06.

- On dosing days: Note that the patient dose must be calculated based on the patient's weight on the day of dosing. Starting dose level for C1D1, dose modification rules and titration rules must be reviewed and implemented prior to dosing as required per protocol (see Sections 10.7, 10.8, 10.9). If a dose delay is required per the dose modification rules (Section 10.8), the patient will not be dosed. The patient will return weekly for assessment of hematology results and AEs until the patient is eligible to receive the next dose of ACE-536 and start the next cycle. The patient should resume the study at the planned dosing cycle (e.g. if the patient missed a dose at C4D1, then they would resume dosing at C4D1 and not skip to C5D1).
- The hematology results can be collected up to 24 hours prior to the dosing day. For patients with treatment interruption, the hemoglobin should be available to confirm eligibility within one day prior to C1D1.
- Patients should be monitored for AEs throughout the study. 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 should include an optional evaluation of gonadal size in male patients at timepoints specified in the Schedule of Events (Section 2).
- If at any visit the systolic blood pressure is $\geq$ 150 mmHg, the diastolic blood pressure is $\geq$ 95 mmHg and/or the absolute increase in either measure from baseline is $\geq$ 20 mmHg, perform one repeat of the blood pressure assessment after a minimum of 15 minutes.
- Patients with clinical signs of a change in spleen size or abnormality should have an abdominal ultrasound or MRI performed as needed throughout the study.
- During treatment, if the pre-transfusion hemoglobin level is increased by $\geq$ 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).
- Cycles 6 through 17: Procedures and visits for Cycles 6 through 9, 10 through 13, and 14 through 17 follow the same procedures and visits as scheduled for Cycles 2 through 5, with some exceptions as noted in the Schedule of Events (Section 2).

- Cycles 24 through 87: Patients may not exceed 87 cycles or 1825 days after first dose, whichever occurs first. The last dose of ACE-536 may not be administered after Day 1825, regardless of the number of cycles completed. Procedures and visits for Cycles 24 through 27, 28 through 31, 32 through 35, 36 through 39, 40 through 43, 44 through 47, 48 through 51, 52 through 55, 56 through 59, 60 through 63, 64 through 67, 68 through 71, 72 through 75, 76 through 79, 80 through 83 and 84 through 87 follow the same procedures and visits as scheduled for Cycles 20 through 23, with some exceptions as noted in the Schedule of Events (Section 2).

12.3. End of Treatment Visit

- The EOT visit should occur 28 days (± 7 days) after the last dose of ACE-536.
- 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.
- Patients who discontinue treatment early should complete the EOT visit at the time of discontinuation.

12.4. Post-Treatment Follow-Up Visit

- The PTFU visit should occur 2 months (± 7 days) after the last dose of ACE-536.
- If a patient has a positive 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.

12.5. Long-Term Follow-Up Visits

- LTFU visits should occur beginning every 6 months (± 14 days) after the last dose of ACE-536 for 3 years after the last dose of ACE-536.

12.6. End of Study Visit

- The EOS visit should occur approximately 3 years after the last dose of ACE-536.
- Physical exam should include an optional evaluation of gonadal size of male patients.

12.7. 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 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 will be provided as either a sterile liquid formulation or a lyophilized powder formulation for reconstitution with water. Each single-use vial of the liquid formulation contains 25 mg of ACE-536 in a 0.5 mL Tris-buffered saline solution (50 mg/mL). Each single-use vial of the lyophilic formulation may contain either 25 mg, 50 mg, or 75 mg ACE-536 for reconstitution with 0.5 mL, 1 mL, or 1.5 mL water, respectively, to form a citrate-buffered solution (50 mg/mL) for injection.

13.3. Study Drug Storage

ACE-536 liquid formulation is stored frozen at ≤ -65°C until use.

ACE-536 lyophilic formulation is stored at 2-8°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 4 injections will be administered per dose.

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 product composition, drug handling, administration, storage, and disposal instructions.

14. ASSESSMENT OF SAFETY

14.1. Adverse Event Definitions

Adverse Event

An 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, Electrocardiogram (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 IB.

Events Not to Be Considered as Adverse Events

Pre-existing medical conditions/signs/symptoms present before the screening period (from study A536-04 for patients without treatment interruption; from study A536-06 for patients with treatment interruption) 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 AEs.

Serious Adverse Event

An 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.

Adverse Events of Special Interest

Events of new malignancy and premalignant lesions (excluding benign tumors or benign neoplasia) are to be reported as described in Section 14.7 within 24 hours of the investigator's knowledge of the event by fax, or other appropriate method, using the SAE Report Form, and must be considered an "Important Medical Event" even if no other serious criteria apply; these events must also be documented in the appropriate page(s) of the CRF and subject's source documents. Documentation of the diagnosed malignancy must be provided at the time of reporting as a serious adverse event (e.g., any confirmatory histology or cytology results, X-rays, CT scans, etc.).

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

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

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

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

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 NCI-CTCAE v4.0, current minor version, preferentially using the graded scales. If there is a change in severity of an AE, 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 AEs 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 patients or observed during or following the course of investigational product administration on the appropriate CRF page. For patients

without treatment interruption, any AE ongoing at the time of EOT in A536-04 needs to be re-entered as an AE in A536-06. For patients with treatment interruption, all non-serious AEs occurring after signing of the ICF until a patient is dosed on C1D1 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 (EOS 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 (EOS 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, 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 ICH and 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

Analysis Populations:

The modified Intent-to-Treat (mITT) Population: All patients enrolled in the study who received at least one dose of ACE-536.

Efficacy Evaluable (EE) Population: All patients administered at least one dose of ACE-536 and have 1) at least three hemoglobin assessments over an 8-week period, not influenced by transfusion, in NTD patients, OR, 2) at least 8 consecutive weeks of transfusion frequency data in TD patients.

Pharmacokinetics Population: All patients who received at least 1 dose of ACE-536 during Study A536-06 and have sufficient serum ACE-536 values 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, SD, median, and range).

15.2.2. Primary Endpoint Analysis

To assess clinical safety, all AEs, vital sign measurements and clinical laboratory information will be summarized. AEs 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. Disposition rates will be summarized.

15.2.3. Secondary Analysis

All efficacy analyses will be performed for the EE and mITT populations.

Efficacy will be evaluated by determining the proportion of β-thalassemia patients who have an erythroid response, defined as 1) a mean hemoglobin increase ≥ 1.0 g/dL or ≥ 1.5 g/dL over an 8-week or 12-week period as compared to baseline, not influenced by RBC transfusion in NTD patients or 2) ≥ 20% or ≥ 50% reduction in RBC transfusion burden over an 8-week or 12-week period as compared to the 8-week or 12-week period pretreatment in TD patients.

Baseline hemoglobin for NTD patients will be the average of two or more measurements performed during the screening period for base study A536-04 (for patients without treatment

interruption) or study A536-06 (for patients with treatment interruption). Hemoglobin measurements within 2 weeks following RBC transfusion will be excluded.

Transfusion burden for TD patients will be defined as the ratio of RBC transfusion units (or mLs) transfused during an interval divided by the duration 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 8 or 12 weeks prior to Cycle 1 Day 1 of base study A536-04 (will also be analyzed using pretreatment period of study A536-06 for patients with treatment interruption). An interval during the treatment plus follow-up period will be defined as an 8-week or 12-week interval after Cycle 1 Day 1 of study A536-06.

The erythroid response, as defined above, will also be evaluated using other pre-treatment and on-treatment intervals and other % reductions in RBC units transfused, including 100% (transfusion-free).

The erythroid response will be summarized using both a point estimate and its exact 95% CI based on binomial distribution.

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

Analysis of other secondary efficacy/PD endpoints will include:

- Time to erythroid response and duration of erythroid response
- Change/percent change from pretreatment in mean transfusion burden in TD patients
- Change in mean hemoglobin level in NTD patients
- Change in pre-transfusion hemoglobin levels in TD patients (including analysis of patient subset with < 20% change in transfusion burden)
- Change from baseline in erythropoiesis parameters, including EPO, reticulocytes, nucleated RBCs, and soluble transferrin receptor
- Change from baseline in hemolysis parameters, including total/indirect bilirubin, and LDH
- Change from baseline in iron overload and iron metabolism parameters, including serum iron, total iron binding capacity (TIBC), transferrin, calculated transferrin saturation, soluble transferrin receptor, ferritin, non-transferrin bound iron (NTBI), hepcidin, iron chelation therapy use and LIC by MRI

The exploratory endpoints will include evaluation of biomarkers related to the TGF-β superfamily, quality of life, change in EMH mass size, change in spleen size, change in BMD, 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% CI.

15.2.4. Pharmacokinetics Analysis

PK parameters for ACE-536, such as maximum serum concentration (C_{max}), time to maximum concentration (t_{max}), and area under the concentration/time curve (AUC), will be estimated using non-compartmental and/or single compartment analysis, as appropriate. Dose proportionality may be assessed using the exposure data (e.g., C_{max}, AUC) 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, as 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 ADA on ACE-536 PK and drug exposure if ADA tests are deemed positive.

15.3. Determination of Sample Size

There is no formal sample size calculation for the study although up to 72 patients may participate from the A536-04 study.

15.4. Interim Analysis

Safety and erythroid response data will be reviewed periodically throughout the study. A formal interim analysis of the safety and efficacy data will be performed to support registration filings.

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 the United States Food and Drug Administration (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 (i.e., 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 U.S. Law. This Web site will not include information that can identify patients, but 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

CRFs 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: National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE)

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