Official Protocol Title:	An Open-Label Extension Study to Evaluate the Long-Term Effects of ACE-536 for the Treatment of Anemia in Patients with Low or Intermediate-1 Risk Myelodysplastic Syndromes (MDS) Previously Enrolled in Study A536-03
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Statistical Analysis Plan

An Open-Label Extension Study to Evaluate the Long-Term Effects of ACE-536 for the Treatment of Anemia in Patients with Low or Intermediate-1 Risk Myelodysplastic Syndromes (MDS) Previously Enrolled in Study A536-03

Sponsor:	Acceleron Pharma Inc. 128 Sidney Street Cambridge, MA 02139 USA Tel: PPD Fax: PPD
Investigational Product:	Luspatercept (ACE-536)
Protocol Number:	A536-05
EudraCT Number:	2014-001280-13
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Date:	08 May 2020

ACCELERON PHARMA SIGNATURE PAGE

The undersigned have approved this Statistical Analysis Plan for use in this study.

Signature: PPD **Date:** 05 MAY 2020

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1. INTRODUCTION

This statistical analysis plan (SAP) describes the statistical methods to be used for the analysis of Acceleron Protocol A536-05. This SAP should be read in conjunction with the study protocol and electronic case report form (eCRF). This version of the plan is intended for the final CSR based on the protocol amendment 04 dated 06 March 2018. Any further changes to the protocol may necessitate updates to the SAP.

The SAP will be signed off before the study database lock. Any deviations from the SAP will be described and justified in the final clinical study report (CSR).

2. STUDY OBJECTIVES

Primary Objective:

- To evaluate the long-term safety and tolerability of ACE-536 in patients with low or intermediate-1 risk MDS who were previously enrolled in study A536-03.

Secondary Objectives:

- To evaluate erythroid response (modified HI-E from International Working Group [IWG] 2006 criteria), defined as proportion of patients with:
  - A mean hemoglobin (Hgb) increase ≥ 1.5 g/dL over an 8-week period as compared to baseline, not influenced by red blood cell (RBC) transfusion, in non-transfusion dependent (LTB) patients (refer to Section 5.1 for definitions of LTB and HTB).
  - A decrease of ≥ 4 units or ≥ 50% of units of RBCs transfused over a period of 8 weeks, relative to the 8 weeks immediately prior to Day 1, in transfusion dependent (HTB) patients.
- To evaluate rates of erythroid, neutrophil and platelet (HI-E, HI-N and HI-P) responses (IWG 2006 criteria)
- To evaluate the rate of RBC transfusion independence lasting ≥ 8 weeks in HTB patients
- To evaluate time to HI-E response and duration of HI-E response (modified and non-modified IWG 2006 criteria)
- To evaluate the mean change in RBC transfusion burden in HTB patients and mean change in hemoglobin levels in LTB patients
- To evaluate the pharmacokinetic (PK) profile of ACE-536
- To evaluate other pharmacodynamic (PD) effects (e.g., iron overload/metabolism, erythropoietin [EPO], and reticulocytes)

Exploratory Objectives:

- To examine biomarkers related to the TGF-β superfamily

- To examine self-reported quality of life using tools including but not limited to the FACT-An questionnaire

3. OVERALL STUDY DESIGN

This open-label extension study will evaluate the safety, tolerability, and PD effects of up to 60 months of ACE-536 treatment in patients with low or intermediate-1 risk MDS previously treated with ACE-536 for up to 3 months in study A536-03.

3.1. Study Design

Patients in all cohorts will receive ACE-536, administered subcutaneously (SC), every 3 weeks for up to 87 cycles or up to 1825 days, whichever occurs first. Dose titration(s), delay(s) and dose reduction(s) may be required for individual patients as outlined in the Individual Dose Modification Rules (protocol Section 10.8).

The base study A536-03 is a phase 2, open-label, ascending dose study to evaluate the effects of ACE-536 on anemia in patients with low or intermediate-1 risk MDS who are not currently receiving treatment with an erythropoiesis-stimulating agent (ESA). A total of up to 153 patients may be enrolled in the base study A536-03 and may be eligible for study A536-05.

Consenting patients that meet the A536-05 eligibility criteria may immediately roll over from A536-03 to study A536-05. These patients will forego the Post-Treatment Follow-up (PTFU) and End of Study (EOS) visits of A536-03 to begin study A536-05. For these patients, C1D1 of A536-05 may take place 28 (± 7) days after the last dose administered in the base study A536-03, which may coincide with the patient's A536-03 End of Treatment (EOT) visit. These patients will be considered "patients without treatment interruption." Patients enrolled in expansion cohorts 2 and 3 in Study A536-03 must meet the "patients without treatment interruption" criteria to be considered for the A536-05 study, unless otherwise prospectively approved by the sponsor.

Patients who complete the EOS visit for the base study A536-03 prior to C1D1 of A536-05 are considered "patients with treatment interruption." These patients will have a 28-day screening period to allow them to be re-assessed for eligibility by meeting additional inclusion criteria, as defined in protocol Section 9.2.

Patients who have completed the EOT visit for the base study A536-03 but have not reached the EOS visit (i.e., patients in the follow-up period for A536-03), may still participate, but should be treated as patients with treatment interruption and should not begin study A536-05 C1D1 until they have completed their A536-03 EOS visit so that new baseline assessments can be measured. Patients transfusion status (LTB or HTB) are defined in Section 5.1. Each HTB patient will have a "pre-transfusion hemoglobin threshold" for requiring transfusion, 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 A536-03 for patients without treatment interruption, or prior to C1D1 of study A536-05 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 should not be transfused if hemoglobin is ≥ 9 g/dL unless indicated for symptoms related to anemia or other reasons at the investigator's discretion.

Patients will participate in the extension study A536-05 for approximately 8 years, including a 28-day screening period, a 60-month treatment period, and a 3-year follow-up period.

A patient without treatment interruption may continue to be dosed with ACE-536 at the same dose level administered at their last dose in study A536-03, unless a dose reduction is required based upon patient dose modification rules from study A536-05.

All patients with treatment interruption will be initially treated with ACE-536 at an assigned starting dose level of 1.0 mg/kg, which has been deemed to be safe, well tolerated, and at least minimally effective by the Safety Review Team (SRT) based on data from the base study A536-03. Examples of possible starting dose levels with respective dose levels for modification (reductions and titrations) are shown in the table below for reference.

Possible Starting Dose Levels with Dose Level Modifications (Reductions and Titrations)

3rd Dose Reduction	2nd Dose Reduction	1st Dose Reduction	Starting Dose Level	1st Dose Titration	2nd Dose Titration	3rd Dose Titration
	0.125 mg/kg	0.25 mg/kg	**0.50 mg/kg**	0.75 mg/kg	1.0 mg/kg	1.33 mg/kg
0.125 mg/kg	0.25 mg/kg	0.5 mg/kg	**0.75 mg/kg**	1.0 mg/kg	1.33 mg/kg	1.75 mg/kg
0.25 mg/kg	0.5 mg/kg	0.75 mg/kg	**1.0 mg/kg**	1.33 mg/kg	1.75 mg/kg	
0.5 mg/kg	0.75 mg/kg	1.0 mg/kg	**1.33 mg/kg**	1.75 mg/kg		
0.75 mg/kg	1.0 mg/kg	1.33 mg/kg	**1.75 mg/kg**			

- Starting dose level for patients with treatment interruption is 1.0 mg/kg.
- Starting dose level for patients without treatment interruption will be last dose level administered in study A536-03 unless a dose reduction is required based upon patient dose modification rules from study A536-05.
- 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, PTFU, LTFU, and EOS visits.
- The maximum dose titration will not exceed 1.75 mg/kg.

3.2. Treatment Discontinuation

Patients will be informed that they have the right to discontinue treatment and/or 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., ESA)
- Medical reason or adverse event, at the discretion of the investigator and/or the medical monitor
- Lack of effect (e.g., worsening anemia for LTB patients as evidenced by sustained reduction in Hgb by ≥ 2 g/dL over 8 weeks or transfusion dependence), to be discussed with the medical monitor
- Disease progression (per IWG criteria for altering natural history of MDS [Cheson et al, 2006]):
  - For patients with 5-10% blasts, a 2nd bone marrow sample should be collected within 4 weeks for clinical assessment (e.g., cytomorphology, cytogenetics) to confirm progression before discontinuing patients from treatment)
- Persistent increase in white blood cell (WBC) count as per Individual Dose Modifications in protocol Section 10.8
- Presence of ≥ 1% blasts in peripheral blood as per Individual Dose Modifications in protocol Section 10.8
- 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/or 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 EOT follow-up visit at the time of discontinuation, then complete the Post-Treatment Follow-up (PTFU) visit 2 months ± 7 days after the last dose of ACE-536, the LTFU visits every 3 months, and finally the EOS visit 3 years after the last dose of ACE-536.

3.3. Sample Size

There is no formal sample size calculation for the study although up to 153 patients may participate from the A536-03 study.

4. ANALYSIS POPULATIONS

4.1. Intent-to-Treat (ITT) Population

The intent-to-treat (ITT) population will consist of all patients who have received at least 1 dose of ACE-536 in A536-05 study. This population will be used for all efficacy analyses.

For patients who directly rolled over from A536-03 study, both A536-03 study and A536-05 study data will be included for the analysis.

For interrupted patients, only A536-05 study data will be included for the analysis.

4.2. Safety Population

Same as ITT population.

4.3. Pharmacokinetics (PK) Population

The pharmacokinetics population will include all patients who have received at least 1 dose of ACE-536 during Study A536-05 and have sufficient serum ACE-536 values for PK analysis.

5. STATISTICAL METHODOLOGY

5.1. Definitions

Transfusion Status

For patients without treatment interruption, transfusion status (LTB or HTB) will carry-over from the base study A536-03. For patients with treatment interruption, transfusion status will be reassessed prior to C1D1 of study A536-05. Transfusion status for all patients is defined as follows for all patients:

- LTB patients are defined as patients who require a transfusion of < 4 units of RBCs in the 8 weeks prior to C1D1
- HTB patients are defined as patients who require a transfusion of ≥ 4 units of RBCs in the 8 weeks prior to C1D1

Baseline

For patients who roll over to Study A536-05 without interruption, the baseline for Study A536-03 will be used as the baseline for Study A536-05.

For patients who enter Study A536-05 with interruption, the last value on or before the first dose of Study A536-05 will be considered as the baseline unless otherwise specified. For baseline assessments that are not scheduled in A536-05 study or data collection is inadequate at the baseline of Study A536-05, the baseline value for Study A536-03 will be used as the baseline for Study A536-05.

Baseline Hemoglobin

Baseline hemoglobin will be an average of hemoglobin measurements within 28 days of Cycle 1 Day 1, excluding measurements within 7 days following RBC transfusion.

Baseline Transfusion Burden

Baseline transfusion burden will be calculated as the total amount of RBC transfusion during the 8 weeks prior to Cycle 1 Day 1.

Baseline EPO and Other Lab Parameters

Baseline erythropoietin (EPO) is defined as the maximum test value within 28 days of Cycle 1 Day 1.

For all other lab parameters, baseline is defined as the last observation on or prior to Cycle 1 Day 1.

End of Treatment (EOT)

Procedures and evaluations for the end of treatment visit should be performed 28 days (±7 days) after the last dose of ACE-536.

Post Treatment Follow-Up (PTFU)

Procedures and evaluations for the post treatment follow-up visit should be performed 2 months (± 7 days) after the last dose of ACE-536.

Long-Term Follow-Up (LTFU)

LTFU visits should occur every 3 months (± 7 days) for 3 years after the last dose of ACE-536.

End of Study (EOS)

Procedures and evaluations for the end of study visit should be performed approximately 3 years after the last dose of ACE-536).

- Patients who discontinue treatment early should complete the EOS visit approximately 3 years after the EOT visit.
- 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.

5.2. General Considerations

Unless otherwise noted, continuous data will be summarized with the following descriptive statistics: number of observations (n), mean, standard deviation (STD), median, minimum and maximum. Categorical data will be summarized with frequencies (n) and percentages (%). In cases where missing data cause percentages not to sum to 100, a missing data row will be provided. Percentages will use column totals as the denominator unless otherwise indicated. For time to event variables, the Kaplan-Meier curves will be presented if the number of patients is more than 5.

All study data will be included in study data listings. Missing data will generally not be imputed, unless otherwise stated.

Data summaries may also be presented for LTB and HTB patients separately as specified. All summaries will be descriptive. No formal hypothesis testing is planned.

5.3. Disposition of Patients

The number and percentage of patients receiving study treatment who completed the treatment period and study period along with the associated reasons for discontinuation from treatment and/or withdrawal from study will be presented.

5.4. Demographic, Baseline Characteristics, and Disease History

The following baseline and demographic characteristics will be summarized by descriptive statistics for the ITT population:

- Race, ethnicity, age, sex, height, weight
- Prior MDS Therapies, time from diagnosis; Prior erythropoiesis stimulating agents (ESA); IPSS and IPSS-R risk group; WHO Subtypes; ICT use
- Baseline ECOG performance status
- Baseline transfusion status (LTB or HTB)
- Baseline hemoglobin and baseline transfusion burden
- Baseline erythropoietin (EPO) and baseline serum ferritin
- RS and SF3B1 mutation status; any splicing factor (yes or no)
- Baseline bone marrow blasts

Demographic and baseline data, medical history, and disease history data will also be listed for each patient.

5.5. Medical History

Medical history will be coded using MedDRA Version 20.0. Summary of medical history will be tabulated by system organ class (SOC) alone and by both SOC and the preferred term (PT).

5.6. Study Drug Exposure

Study drug exposure will be descriptively summarized for the safety population and will present the duration of exposure, the number of treatment cycles, the total dose administered, the number of patients with dose delay and reduction, and the number of patients with dose increase.

The duration of exposure will be calculated as (last dose date – first dose date) + 21.

The total number of cycles will be summarized by presenting the number and percentage of patients in each category.

The total dose administered is the total amount of study drug in mg a patient received during the treatment period.

Study drug administration details will be listed for each patient.

5.7. Prior and Concomitant Medication and Procedures

5.7.1. Prior and Concomitant Medication

The prior and concomitant medications are coded with WhoDrugDDEB2 201209. The medications will be presented for the safety population.

Medications will be assigned as prior or concomitant based on the following rules:

- If both the start and stop date exist and are before the first dose date of study drug, the medication will be counted as prior.
- If the start date is on or after the first dose date of study drug, the medication will be counted as concomitant.
- If the start date is before the first dose date of study drug and the stop date is after the first dose date of study drug or the medication is ongoing, the medication will be counted as prior and concomitant.
- If the start date is missing and the stop date is before the first dose of study drug, the medication will be counted as prior.
- If the start date is missing and the stop date is after the first dose of study drug or the medication is ongoing, the medication will be counted as concomitant.
- If the start and stop dates are missing, the medication will be counted as concomitant.

Prior and concomitant medications will be summarized separately.

5.7.2. Non-Medication Procedures

All non-medication procedures will be listed for each patient.

5.8. Efficacy and Safety Analyses

No formal hypothesis testing is planned. All efficacy analyses will be performed using the ITT population.

In general, the below rules apply to the derivations of efficacy endpoints related to hemoglobin and RBC transfusion data unless specified otherwise:

- Hemoglobin measurements within 7 days following RBC transfusion will be excluded from the efficacy analysis
- For each patient, all efficacy endpoints will be derived based on an analysis cutoff day, defined as the last dose + 56 days or the last date from transfusion record data, whichever is earlier.

5.8.1. Primary Efficacy Endpoint

Since the primary objective of this study is to evaluate the long-term safety and tolerability of ACE-536 in patients with low or intermediate-1 risk MDS who were previously enrolled in study A536-03, there is no primary efficacy endpoint specified for this study.

5.8.2. Secondary Efficacy Endpoints

5.8.2.1. Erythroid Response (modified HI-E from IWG 2006 criteria)

Erythroid response (modified HI-E from IWG 2006 criteria) is defined as the proportion of patients with:

- A mean hemoglobin (Hgb) increase ≥ 1.5 g/dL over an 8-week period as compared to baseline, not influenced by RBC transfusion, in LTB patients.
- A decrease of ≥ 4 units or ≥ 50% of units of RBCs transfused over a period of 8 weeks, relative to the 8 weeks immediately prior to Day 1, in HTB patients.

A point estimate of the proportion of patients achieving a modified erythroid response will be presented along with its associated exact 95% confidence interval based on binomial distribution.

The complete hemoglobin measurements and RBC transfusion records will be listed for each patient.

5.8.2.2. RBC Transfusion Independence

For patients with ≥ 2 units of RBC transfusion at baseline, RBC transfusion independence (RBC-TI) response is defined as not requiring RBC transfusion for 8 or more weeks while on treatment.

The proportion of RBC-TI responders will be estimated by a point estimate along with the exact 95% confidence interval based on binomial distribution. The denominator is the number of patients with ≥ 2 units of RBC transfusion at baseline.

Time to RBC-TI will be defined as the time from the first dose date to the starting date of the first consecutive 8-week interval not requiring RBC transfusion. Duration of RBC-TI will be calculated as the longest period of time not requiring RBC transfusion during treatment and will be censored at the analysis cutoff date for ongoing responses.

Both time to and duration of RBC-TI will be analyzed as continuous variables and summarized by descriptive statistics for RBC-TI responders only. Duration of RBC-TI will also be analyzed as a time to event variable using Kaplan-Meier method.

The derivations of RBC-TI response, time to and duration of RBC-TI will be listed for each patient.

RBC-TI responders may experience multiple periods of ≥8 weeks of RBC-TI response. For all RBC-TI responders, the number of episodes and cumulative duration will be summarized. As an exploratory analysis, the cumulative duration of the multiple responses will be analyzed using Kaplan-Meier method.

5.8.2.3. HI-E Response Rate

Erythroid (HI-E) response will be defined following IWG 2006 criteria.

- For low transfusion burden (LTB) patients, a hemoglobin increase of ≥ 1.5 g/dL from baseline during any rolling 8-week period in the absence of transfusion. The rolling 8-week period starts and ends at a hemoglobin measurement date with duration ≥ 8 weeks.

- For high transfusion burden (HTB) patients, a reduction by ≥ 4 RBC units transfused during any rolling 8-week interval on treatment compared to baseline.

A point estimate of the proportion of HI-E responders will be presented along with its associated exact 95% confidence interval based on binomial distribution. HI-E response will also be listed for each patient.

There are two versions of a HI-E response for LTB patients.

- All hemoglobin values increase of ≥ 1.5 g/dL from baseline during any rolling 8-week period in the absence of transfusion.
- Mean hemoglobin value increase of ≥ 1.5 g/dL from baseline during any rolling 8-week period in the absence of transfusion.

5.8.2.4. HI-P and HI-N Response Rate

Platelet (HI-P) and neutrophil (HI-N) response will be defined for patients with abnormal baseline values, following IWG 2006 criteria.

HI-P response will be defined for patients with baseline platelet count < 100×10^9/L:

- For patients with baseline ≥ 20×10^9/L, an absolute mean increase of ≥ 30×10^9/L during any rolling 8-week interval on treatment;
- For patients with baseline < 20×10^9/L, mean value of > 20×10^9/L and mean percentage increase ≥ 100% during any rolling 8-week interval on treatment.

HI-N response will be defined for patients with baseline neutrophil count (ANC) < 1.0×10^9/L:

- Mean percentage increase ≥ 100% and an absolute mean increase > 0.5×10^9/L during any rolling 8-week interval on treatment compared with baseline.

The number and percentage of HI-N/HI-P responders will be presented. The HI-N and HI-P response will also be listed for each patient.

5.8.2.5. Time to and Duration of HI-E Response

Time to and duration of HI-E response will be analyzed for HI-E responders only.

Time to HI-E response will be defined as the time from the first dose date to the first date of any rolling 8-week interval achieving HI-E response.

Duration of HI-E response will be defined as below for LTB and HTB patients, respectively:

- For LTB patients, duration of HI-E response will be calculated as the longest interval during which all hemoglobin measurements have an increase of ≥ 1.5 g/dL from baseline.
- For HTB patients, duration of HI-E response will be calculated for the longest such interval as the time from the start date of the first rolling 8-week interval achieving hematologic response to the end date of the last consecutive rolling 8-week interval achieving response.

When there are multiple disjoint intervals with response, the longest interval will be used. Patients with response ongoing by the analysis cutoff day will be censored at that date.

Both time to and duration of HI-E response will be analyzed as continuous variables and summarized by descriptive statistics. In addition, duration of HI-E response will also be analyzed as a time to event endpoint. The survival curves will be estimated using Kaplan-Meier method if the number of patients is more than 5.

HI-E responders may experience multiple periods of ≥ 8 weeks of HI-E response. For all HI-E responders, the number of episodes and cumulative duration will be summarized. As an exploratory analysis, the cumulative duration of the multiple responses will be analyzed using Kaplan-Meier method.

5.8.2.6. Iron-Related Parameters

Iron metabolism parameters including serum iron, total iron binding capacity (TIBC), transferrin, soluble transferrin receptor, ferritin, and hepcidin will be taken at Day 1 of Cycle 1 and every 4 subsequent cycles, EOT, PTFU and EOS visits. Descriptive statistics of observed values, absolute and percentage change from baseline values at each postbaseline visit will be presented.

Shift analyses will also be performed to summarize serum ferritin EOT change from baseline. Serum ferritin will be grouped into three categories: < 300, ≥ 300 and < 1000, ≥ 1000 (μg/L).

Plots of iron parameters for both mean observed values and mean absolute change from baseline over time will be presented.

5.8.2.7. Erythropoietin and Immature RBC Parameters

Blood samples relating to erythropoietin parameters including serum erythropoietin levels, reticulocytes and nucleated RBCs will be taken at Day 1 of Cycle 1 and every 4 subsequent cycles, EOT, PTFU and EOS visits. Descriptive statistics of observed values, absolute and percentage change from baseline values at each postbaseline visit will be presented. Plots of erythropoietin parameters for mean observed values, mean change and mean percentage change from baseline over time will be presented.

5.8.2.8. Bone Metabolism Parameters

Bone metabolism parameters including bone specific alkaline phosphatase (BSAP) and serum C-telopeptide of type I collagen (CTX) will be taken at Cycle 1 Day 1, Cycle 4 Day 1, and EOT visits. Descriptive statistics of observed values, absolute and percentage change from baseline values at each postbaseline visit will be presented.

5.8.2.9. Hemolysis-Related Parameters

Hemolysis-related parameters include direct bilirubin, total bilirubin, and lactate dehydrogenase (LDH). Descriptive statistics of observed values, absolute and percentage change from baseline values at each postbaseline visit will be presented. Plots of hemolysis-related parameters for both mean observed values, mean change and mean percentage change from baseline over time will be presented.

5.8.2.10. Other Analysis

For the following continuous variables, the descriptive statistics including mean, standard deviation, median and range will be presented:

- Maximum reduction over rolling 8-week intervals (absolute and percentage) in transfusion burden from baseline in HTB patients
- Maximum increase in hemoglobin level from baseline in LTB patients
- Maximum hemoglobin mean change from baseline during any rolling 8-week period in LTB patients
- Maximum decrease/percentage decrease in serum ferritin
- Change in absolute reticulocytes
- Change in pre-transfusion hemoglobin level in HTB patients
- Frequency of RBC transfusions in HTB patients

5.8.3. Exploratory Endpoints

5.8.3.1. FACT-An Questionnaire

The FACT-An Questionnaire will be completed at Day 1 of Cycle 1 and every 4 subsequent cycles, End of Treatment, and End of Study visit. The FACT-An questionnaire contains 47 questions which are divided into the following subscales:

- Physical Well-Being (PWB) (7 questions: Item Score range 0 - 28)
- Social/Family Well-Being (SWB) (7 questions: Item Score range 0 - 28)
- Emotional Well-Being (EWB) (6 questions: Item Score range 0 - 24)
- Functional Well-Being (FWB) (7 questions: Item Score range 0 - 28)
- Anemia subscale (AnS) (20 questions: Item Score range 0 - 80)

Patients give individual responses to each question on a scale of 0 to 4 (0=Not at all; 1=A little bit; 2=Somewhat; 3=Quite a bit; 4=Very much). Item scores in the PWB and EWB subscales will be derived by subtracting the response value from 4. Similarly, all item scores in the AnS subscale except Item Codes An5, An7, BL4 and An13[3] will be derived by subtracting the response value from 4. Thus, a higher Item Score indicates a better quality of life. Subscale totals will be derived as follows:

$$\frac{\text{Sum of Item Scores x Number of Items in Subscale}}{\text{Number of Items Answered}}$$

For example, if 6 questions are answered in the Physical Well-Being subscale and the Item scores sum to a total score of 18, then the subscale score will be (18 x 7)/6 = 21.

If 50% or more of responses in any subscale are missing, the subscale score will be set to missing.

The following total scores will also be derived:

FACT-An Total Outcome Index (TOI) derived as: PWB + FWB + AnS
(Score range 0 – 136)

FACT-G Total Score (FACT-G) derived as: PWB + SWB + EWB + FWB
(Score range 0 – 108)

FACT-An Total Score (FACT-An) derived as: PWB + SWB + EWB + FWB + AnS
(Score range 0 – 188)

If 20% or more of the responses that contribute to the FACT-G score are missing, the FACT-G score will be set to missing, i.e., at least 22 of the 27 items contributing to FACT-G must be present. Furthermore, FACT-An TOI, FACT-G and FACT-An scores should only be calculated if all component subscales have valid scores. If any subscale total is missing, the respective total scores to which the subscale contributes will also be set to missing.

Individual subscale scores and total scores will be summarized by visit. All derived subscale and total scores and all individual responses will be listed.

The association between FACT-An improvement and RBC-TI response will also be explored.

5.8.4. Subgroup Analyses

Subgroup analyses of the efficacy endpoints will be performed for the following baseline parameters:

- RS status (positive or negative),
- SF3B1 mutation status (mutated or wild type)
- Splicing factor mutation status (mutated or wild type)
- Baseline EPO level (≤ or >100, ≤ or > 200; ≤ or >500)
- Baseline bone marrow blasts (< 5% or ≥ 5%)
- IPSS risk group (low, Int-1, Int-2, or high)
- IPSS-R risk group (very low, low, intermediate, high, very high)

Logistic regression analysis will be performed to evaluate the effect of following prognostic factors on the IWG HI-E response and RBC-TI individually.

- IPSS risk group (low vs Int-1/Int-2/high)
- Baseline EPO (continuous)
- Baseline EPO level (≤ 100 vs. > 100 IU/L)
- Baseline EPO level (≤ 200 vs. > 200 IU/L)
- Prior ICT (Yes vs. No)
- Prior ESA (Yes vs. No)
- RS status (positive vs. negative),
- SF3B1 mutation status (mutated vs wild type)

Multivariable logistic regression analysis with backward selection will be performed to evaluate the association of association between baseline factors and IWG HI-E and RBC-TI response.

5.8.5. Safety Analyses

The safety endpoints will be summarized based on the Safety Population. The safety endpoints include the incidence of treatment emergent adverse events, changes in laboratory tests, vital signs and ECG's.

Adverse events (AEs) will be coded using MedDRA Version 20.0. Severity of AEs will be coded using National Cancer Institute Common Toxicity Criteria for Adverse Events version 4.0 (NCI-CTCAE v4.0).

5.8.6. Adverse Events

All AEs and SAEs occurring after the Study A536-03 (for patients without interruption) or Study A536-05 (for patients with interruption) Cycle 1 Day 1 until approximately 2 months after the last study drug administration (PTFU visit) are to be reported and documented on the AE CRF.

Treatment emergent adverse events (TEAE) are defined as:

- AE starting or worsening from the first date of study drug;
- AE occurs on the first date of study drug and the onset check box is marked "Onset after first dose of study drug";
- AE with a missing start date and a non-missing stop date on or after the first dose of study drug;
- AE with both a missing start and stop date.

A drug-related TEAE is defined as any TEAE related to the study medication as assessed by the investigator or with missing assessment of the causal relationship.

The following summaries will also be presented:

- Number and percentage of patients reporting each AE, categorized by SOC and PT
- Number and percentage of patients reporting each AE experienced by ≥ 5% of patients in all patients by PT
- Number and percentage of patients reporting SAE, categorized by SOC and PT
- Number and percentage of patients reporting Grade ≥ 3 AE, categorized by SOC and PT
- Number and percentage of patients reporting related AE, categorized by SOC and PT
- Number and percentage of patients reporting AE leading to drug withdrawal, categorized by SOC and PT
- Number and percentage of patients reporting AEs of interest by cycle

Note that counting will be by patient, not event, and patients are only counted once within each SOC or preferred term. If a patient has the same AE at more than one severity, or with more

than one relationship to study drug, the most severe rating or the stronger causal relationship to study drug will be given precedence. Any missing severity, causality, or outcome will not be imputed and classed as unknown.

All AEs will be listed. The following listings will also be provided: 1) patients with SAEs; 2) patients with Grade ≥ 3 AEs; 3) patients with AEs leading to discontinuation.; and 4) death.

5.8.7. Laboratory Evaluations

Results from the following laboratory parameters, recorded at their respective time points will be summarized by time point:

Hematology (Screening, Day 1 of each cycle, End of Treatment, Post Treatment Follow Up and End of Study): RBC, 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), red blood cell distribution width (RDW), and nucleated RBCs (nRBCs)

Chemistry (Screening, Day 1 of Cycle 1 and Day 1 of every other subsequent cycles, End of Treatment and End of Study): Sodium, potassium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), total bilirubin, direct bilirubin, alkaline phosphatase, blood urea nitrogen (BUN)/urea, creatinine, gamma-glutamyl transpeptidase (GGT), calcium, phosphorus, glucose, amylase, lipase, total protein, albumin, and uric acid

Urine Analysis (Day 1 of Cycle 1 and every 4 subsequent cycles, End of Treatment and End of Study): pH, specific gravity, protein, glucose, ketones, blood, leukocyte esterase, nitrite, microscopic examination (if performed)

Urine Chemistry (Day 1 of Cycle 1 and every 4 subsequent cycles, End of Treatment and End of Study): Microalbumin and creatinine; albumin/creatinine ratio

Categorical and numeric variables will be presented separately. Actual values and changes in hematology and biochemistry laboratory values from baseline will be summarized by time point. Shift tables for the hematology and biochemistry laboratory parameters comparing values above, within and below the normal reference range at baseline to the end of treatment visit will be presented using standard reference ranges.

Peripheral blood smears are taken at Screening, Day 1 of Cycle 1 and every 4 subsequent cycles, and at the end of treatment visit. These data will be listed only.

All laboratory values will be listed for all patients.

5.8.8. Vital Signs

Vital signs parameters include weight (kg), heart rate, systolic and diastolic blood pressure, respiratory rate, and temperature (°C). Vital signs are recorded at Screening and on Days 1 of each cycle and End of Treatment. For each parameter at each time point, the observed values and change from baseline will be summarized. Vital sign data will also be listed for all patients.

5.8.9. ECG Results

12-lead ECG results are recorded at Cycle 1 Day 1 and at the end of treatment visit. The quantitative ECG assessments (Ventricular rate, QRS width, PR interval, and QTcF interval) will be summarized at each time point.

ECG overall interpretation (normal, abnormal/not clinically significant [NCS] and abnormal/clinically significant [CS]) will be presented for actual values and changes from baseline (Screening observation) to each post-baseline visit, expressed as Improvement, No Change, and Deterioration, defined as follow:

- Improvement = CS to Abnormal NCS, Abnormal NCS to Normal
- Deterioration = Normal to Abnormal NCS or CS, Abnormal NCS to Abnormal CS
- No change = Normal to Normal, Abnormal NCS to Abnormal NCS, Abnormal CS to Abnormal CS

If a result is missing for any patient, then an 'Unknown' category will be presented. ECG results will be listed for all patients.

5.8.10. Physical Examination

A physical examination is conducted at Screening, Cycle 1 Day 1 and Day 1 of every 4 cycles and at the end of treatment and end of study visits. Physical exam details will be listed only.

5.8.11. Long-term Follow-up

A listing of Long-term Follow-up data including the visit date, malignancy status, and survival status will be provided.

5.9. Pharmacokinetics Analysis

5.9.1. Pharmacokinetic Sampling Schedule

Blood samples for determination of ACE-536 serum concentrations are collected at Cycle 1 Day 1, Day 1 of every 4 subsequent cycles, EOT, and EOS.

Acceptable time windows for pharmacokinetic blood draws are ± 1 day in Cycle 1 and ± 7 days for the other cycles with PK sampling scheduled.

5.9.2. Data Handling

Concentrations that are below the limit of quantitation (BLQ) prior to the first dose will be assigned a numerical value of zero. Post-treatment concentrations that are BLQ will be treated as missing.

Concentrations assigned a value of missing will be omitted from the descriptive statistics. A concentration value of zero will be excluded from the computation of the geometric mean (geometric CV%). If any patients are found to be noncompliant with respect to dosing, have incomplete data, or encounter other circumstances that would affect the evaluation of pharmacokinetics, a decision will be made on a case-by-case basis as to their inclusion in the

pharmacokinetic analysis. Data excluded from pharmacokinetic analysis will be included in the data listings, but not in the summaries.

In tables and listings for the derived pharmacokinetic data, there should be four decimal places for numerical values below 1, three decimal places for numeric values below 10 but above 1, and two decimal places for numeric values above 10. However, the listings of raw data should not have more decimal places than the actual data.

5.9.3. Pharmacokinetic Analysis

All ACE-536 serum concentrations will be listed by patient and scheduled time (visit and study). Actual dosing/sampling time, sample time relative to first dosing time, visit, and concentration as done for the 03 study will be presented in the listing.

The ACE-536 serum concentrations will be summarized by scheduled time, including N (number of observations), arithmetic mean, arithmetic standard deviation (SD), arithmetic coefficient of variation (CV%), geometric mean, geometric CV%, minimum, median, and maximum. Mean (SD) serum concentration-time profiles will be presented on linear scales.

5.10. Interim Analysis

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

5.11. Protocol Violations or Deviations

Protocol violations and deviations will be reviewed and may result in a patient and/or patient visit data being excluded from the statistical summaries. All decisions regarding exclusion of any patient data will be made and recorded during the final data review.

5.11.1. Violation Criteria

Patients who meet any of the following criteria will be listed:

- Non-compliance with inclusion criteria
- Non-compliance with exclusion criteria
- Unauthorized concomitant therapy
- Less than 8 weeks historical hemoglobin data
- Dose not modified according to Dose Modification Criteria
- Study assessment outside of visit window

5.11.2. Protocol Deviations

Deviations from the protocol, as defined in the protocol, will be documented on an ongoing basis by the study monitors and project manager throughout the study period.

At the time of database lock and while the protocol violations are being reviewed, the project manager will forward all relevant documentation highlighting protocol deviations to the study

statistician. These deviations will be included in the protocol violation document for agreement and will be listed with the protocol violations in the CSR.

5.12. Handling of Missing Data and Visit Window

5.12.1. Missing Data

As a general principle, no imputation of missing data for other variables will be done. Exceptions are the start and stop dates of AEs and concomitant medication. The imputed dates will be used to allocate the medication as prior or concomitant medications and to determine whether an AE is/is not treatment emergent. Listings of the AEs and concomitant medications will present the actual partial dates; imputed dates will not be shown.

5.12.2. Missing Dates for Adverse Event

Imputing partial AE start dates:

- If the year is unknown, the date will not be imputed and will be assigned a missing value.
- If the month is unknown, then:
  - If the year matches the first dose date, then impute the month and day of the first dose date.
  - Otherwise, assign January.
- If the day is unknown, then:
  - If the month and year match the first dose date, then impute the day of the first dose date.
  - Otherwise, assign '01'.

Imputing partial AE stop dates:

- If the year is unknown, the date will not be imputed and will be assigned a missing value.
- If the month is unknown, then assign December.
- If the day is unknown, then assign the last day of the month.

5.12.3. Missing Dates for Concomitant Medication

If start date is missing or partial:

- if month is missing, use January
- if day is missing, use the first day of the month under consideration
- if year is missing, use year of the informed consent date
- if entire date is missing, use informed consent date

If stop date is missing or partial:

- if month is missing, use December
- if day is missing, use the last day of the month under consideration
- if year or the entire date is missing, set to December 31st, 2099

If the imputed start date is after the stop date, then the imputed start date will be one day prior to the stop date.

5.12.4. Missing Dates for Disease Diagnosis Date

For disease diagnosis dates, the imputation rules are

- if day is missing, use 15th of the month
- if both day and month are missing, impute as January 1st
- if month is missing, impute as January
- if year is missing, set to missing

5.13. Changes in Conduct or Planned Analyses from the Protocol

Major changes between SAP and the planned analysis in Protocol are described below:

Item	Difference Between Protocol and SAP
NTD/TD	In SAP, the terms LTB/HTB are used in place of NTD/TD in protocol.
mITT	In the SAP, ITT is used in place of mITT in the protocol.
Secondary objectives: time to and duration of mHI-E	The secondary endpoints time to and duration of mHI-E will not be analyzed.
Efficacy Evaluable Population (EE)	Analysis on Efficacy Evaluable population will not be performed. All analyses are on ITT population
Definition of baseline hemoglobin	Protocol definition: Baseline hemoglobin will be the average of at least two measures (not influenced by transfusion within 7 days of measurement); one measure performed within one day prior to Cycle 1 Day 1 and the other performed 7-28 days prior to Cycle 1 Day 1. This definition is revised in this SAP to reflect the derivation rule implemented for this study.

6. REFERENCES

Cheson BD, Greenberg PL, Bennett JM, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood 2006;108:419-25.

National Cancer Institute Common Toxicity Criteria for Adverse Events, version 4.0 (NCI-CTCAE v4.0)

7. APPENDICES

7.1. Appendix 1: List of Abbreviations

Abbreviation	Definition
AE	Adverse Event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BSAP	Bone specific alkaline phosphatase
BUN	Blood urea nitrogen
CRF	Case report form
CTX	C-telopeptide of type I collagen
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EE	Efficacy Evaluable
ESA	Erythropoiesis stimulating agent
EOS	End of Study
EPO	Erythropoietin
EOT	End of Treatment
FACT-An	Functional Assessment of Cancer Therapy-Anemia Scale
HI-E	Erythroid response
HI-N	Neutrophil response
HI-P	Platelet response
HTB	High Transfusion Burden
ICT	Iron Chelation Therapy
IPSS	International Prognostic Scoring System
IPSS-R	Revised International Prognostic Scoring System
ITT	Intent-to-Treat
IWG	International Working Group
LDH	Lactate dehydrogenase
LTB	Low Transfusion Burden
LTFU	Long-Term Follow-Up
MedDRA	Medical Dictionary for Regulatory Activities
MCH	Mean corpuscular hemoglobin

Abbreviation	Definition
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MDS	Myelodysplastic syndromes
NCI-CTCAE	National Cancer Institute-Common terminology criteria for adverse events
nRBC	Nucleated red blood cells
PD	Pharmacodynamic
PK	Pharmacokinetic
PTFU	Post-treatment Follow-up
QoL	Quality of life
RBC	Red blood cell
RDW	Red blood cell distribution width
RS	Ring Sideroblasts
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SF3B1	Splicing factor 3B subunit 1
SRT	Safety Review Team
TGF-β	Transforming growth factor beta
TIBC	Total iron binding capacity
WBC	White blood cell
WHO	World Health Organization

7.2. Appendix 2: Schedule of Events for Study 03

	Screen	Treatment Period																Follow up period		
		Cycle 1				Cycle 2			Cycle 3			Cycle 4			Cycle 5			EOT[12]	Post-Treatment Follow Up	EOS[13]
		C1D1[2]	C1D8	C1D11	C1D15	C2D1[2,15]	C2D8	C2D15	C3D1[2,15]	C3D8	C3D15	C4D1[2,15]	C4D8	C4D15	C5D1[2,15]	C5D8	C5D15	Day 113 (± 7d)	Day 141 (± 7d)	Day 169 (± 7d)
	Day -28	Day 1	Day 8 (± 1d)	Day 11 (± 1d)	Day 15 (± 1d)	Day[14] 22 (± 2d)	Day 29 (± 2d)	Day 36 (± 2d)	Day[14] 43 (± 2d)	Day 50 (± 2d)	Day 57 (± 2d)	Day[14] 64 (± 2d)	Day 71 (± 2d)	Day 78 (± 2d)	Day[14] 85 (± 2d)	Day 92 (± 2d)	Day 99 (± 2d)			
Informed consent	X																			
Inclusion/Exclusion	X	X																		
Medical history	X																			
FACT-An Questionnaire [18]	X														X			X[19]		X
Physical examination	X	X				X			X			X			X			X		X
Vital signs[1]	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		
ECOG Status	X								X			X						X		X
ECG (12 lead)	X					X												X		
Bone marrow aspirate/biopsy[3]	X								X									X		
Serum iron studies[4]	X	X				X	X		X			X			X			X	X	X
Serum folate and B12	X																			
Erythropoietin levels	X	X	X		X	X						X						X	X	X
Hematology5	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Peripheral blood smear	X	X				X									X			X		
Serum chemistry[6]	X	X			X	X	X		X	X		X	X		X	X		X		X
Urinalysis and Urine Chemistry[7]		X				X						X						X		X
Anti-drug antibody[16]		X										X						X		X[16]
PK collection		X	X	X	X	X	X					X			X	X	X	X	X	X
PD biomarkers[8]		X				X			X			X			X			X		X
Bone biomarkers[9]		X										X						X		
Pregnancy test10	X	X				X			X			X			X			X		X
Evaluate transfusion frequency[11]	X	X				X			X			X[2]			X			X		X
Concomitant medications and AEs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Administer ACE-536[17]		X				X[14]			X[14]			X[14]			X[14]					

[1] **Vital signs:** Weight, heart rate, systolic and diastolic blood pressure, respiration rate and temperature (measured in degrees Celsius). Height is measured only at Screening.
[2] **Study procedures** must be done prior to administration of study drug.
[3] **Bone marrow aspirate/biopsy** including cytogenetics at screening must be performed ≤ 3 months prior to C1D1 for evaluation of patient eligibility and other PD biomarkers to be determined (e.g., GDF15, GDF8, and GDF11). A bone marrow aspirate must be performed within 21 days after C3D1 (a bone marrow biopsy is not required). If hemoglobin increases ≥ 2 g/dL, an optional bone marrow aspirate can be performed within 7 days of the result. At the end of treatment visit, a bone marrow aspirate must be performed (a bone marrow biopsy is optional).
[4] **Iron Studies:** Serum iron, TIBC, transferrin, soluble transferrin receptor, ferritin.
[5] **Hematology:** RBC, WBC with differential, hemoglobin, hematocrit, nRBC, reticulocyte count, platelet count, MCV, MCH, MCHC, and RDW. On dosing days, hemoglobin values are to be drawn and resulted (up to 1 day) prior to dosing (see protocol Section 10.8.1, Patient Dose Modification Rules). Historical hemoglobin data will be collected for 24 weeks, where available, prior to C1D1. During the screening period, hemoglobin will be measured twice; one measure performed within one day prior to Cycle 1 Day 1 and the other performed 7-28 days prior to Cycle 1 Day 1. Neither hemoglobin measure should be influenced by transfusion within 7 days of measurement.
[6] **Chemistry**: Sodium, potassium, AST, ALT, lactate dehydrogenase (LDH), total bilirubin, direct bilirubin, alkaline phosphatase, blood urea nitrogen (BUN)/urea, creatinine, GGT, calcium, phosphorus, glucose, amylase, lipase, total protein, albumin, and uric acid.
[7] **Urinalysis by dipstick analysis (local lab):** pH, specific gravity, protein, glucose, ketones, blood, leukocyte esterase, and nitrite, with microscopic examination if indicated. **Urine Chemistry (central lab):** Microalbumin and creatinine.
[8] **PD Biomarkers:** Hepcidin, GDF15, GDF8, GDF11, and others to be determined.
[9] **Bone Biomarkers:** BSAP and CTX.
[10] **Pregnancy test:** (urine or serum) is required for female patients of child bearing potential at screening and prior to each dose of ACE-536.
[11] **Transfusion history** will be collected for 24 weeks, where available, prior to C1D1.
[12] **End of Treatment (EOT)**: Should be performed 28 days (± 7 days) after the last dose of ACE-536. Patients who discontinue treatment early should complete the end of treatment visit at the time of discontinuation and complete the post-treatment follow-up (PTFU) and EOS follow-up visits 28 days (± 7 days) and 56 days (± 7 days) after the EOT visit.
[13] **End of Study (EOS):** Should be performed 56 days (± 7 days) after the Day 113/EOT visit.
[14] **Day 85 ± 2 days** is the last possible study day that ACE-536 may be administered, regardless of the cycle.
[15] If a **dose delay** is required per the dose modification rules the patient will not be dosed. The patient will return weekly to assess hematology results and adverse events until the patient is eligible to administer the next dose of ACE-536.
[16] If the patient has a **positive ADA** result at their last assessment, the patient may be asked to return approximately every three months for additional testing, until a negative result is obtained or the result is considered stabilized.
[17] For the **first dose of ACE-536**, dosing should occur after a minimum of 7 days post-transfusion and a minimum of 24 hours prior to a scheduled transfusion.
[18] **Expansion cohort** patients only, administration of FACT-An quality of life questionnaire.
[19] FACT-An should be completed at the EOT visit only for patients that discontinue treatment early.

7.3. Appendix 3: Schedule of Events for Study 05

	Screening[1]	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Cycle 7	Cycle 8	Cycle 9
		C1D1[2]	C2D1[2]	C3D1[2]	C4D1[2]	C5D1[2]	C6D1[2]	C7D1[2]	C8D1[2]	C9D1[2]
Informed consent	X[1]									
Inclusion/Exclusion	X	X[2]								
Medical history[3]	X									
QoL Questionnaires [4]		X				X				X
Physical examination	X	X				X				X
Vital signs[5]	X	X	X	X	X	X	X	X	X	X
ECOG Status	X	X		X		X		X		X
ECG (12 lead)[6]		X								
Bone marrow aspirate[7]	X	X[7]								X[7]
Serum iron studies[8]		X				X				X
Erythropoietin levels		X				X				X
Hematology[9]	X	X	X	X	X	X	X	X	X	X
Peripheral blood smear		X				X				X
Serum chemistry[10]	X	X		X		X		X		X
Urinalysis and Urine Chemistry[11]	X	X				X				X
Anti-Drug Antibody[12]		X				X				X
PK collection		X				X				X
PD biomarkers[13]		X				X				X
Pregnancy test[14]	X	X	X	X	X	X	X	X	X	X
Evaluate transfusion frequency[15]	X	X	X	X	X	X	X	X	X	X
Administer ACE-536		X	X	X	X	X	X	X	X	X
Concomitant medications and AEs	Collected Continuously									

	Cycle 10 C10D1[2]	Cycle 11 C11D1[2]	Cycle 12 C12D1[2]	Cycle 13 C13D1[2]	Cycle 14 C14D1[2]	Cycle 15 C15D1[2]	Cycles 16 - 87[2,16]	EOT[17]	PTFU[18]	LTFU[19]	EOS[20]
Informed consent							Repeat Cycles 12-15 eighteen times: visits are 21 ±5 days apart unless a dose delay is required.				
Inclusion/Exclusion											
Medical history[3]											
QoL Questionnaire[4]				(X)[4]				X			
Physical examination				X				X			
Vital signs[5]	X	X	X	X	X	X		X			
ECOG Status		X		X		X		X			
ECG (12 lead)[6]								X			
Bone marrow aspirate[7]				(X)[7]				X[7]			
Serum iron studies[8]				X				X	X		
Erythropoietin levels				X				X	X		
Hematology[9]	X	X	X	X	X	X		X	X		
Peripheral blood smear				X				X			
Serum chemistry[10]		X		X		X		X			
Urinalysis and Urine Chemistry[11]				X				X			
Anti-Drug Antibody[12]				X				X[12]			
PK Collection				X				X			
PD biomarkers[13]				X				X			
Pregnancy test[14]	X	X	X	X	X	X		X			
Evaluate transfusion frequency[15]	X	X	X	X	X	X		X	X		
Administer ACE-536[2]	X	X	X	X	X	X					
Monitoring for AEs of special interest[21] (Refer to Section 14.1 for details)	After signing ICF and until at least 3 years post last dose of IP or until death, loss to follow up, withdrawal of consent for further data collection.										
Survival follow-up[21]										X	X
Concomitant medications and AEs	Collected Continuously										

[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. Patients with treatment interruption will need to qualify per the additional inclusion criteria listed in Section 9.2.1. For patients without treatment interruption, C1D1 may coincide with EOT visit of the base study A536-03. Procedures that are required to confirm eligibility for patients without treatment interruption can be performed at the base study A536-03 EOT visit and used to confirm eligibility prior to dosing on C1D1 of study A536-05.

[2] **Study procedures** must be done prior to administration of study drug. For patients without treatment interruption, C1D1 procedures shaded grey may be conducted as part of the EOT visit for study A536-03 and may not need to be repeated for study A536-05. All screening and Cycle 1 Day 1 procedure results required to confirm eligibility must be obtained and reviewed prior to study drug administration in A536-05.
Visit schedule: Each dosing visit (cycles 1-87) will be 21 days (±5 days) from the previous dosing visit, unless a dose delay is required. If a dose delay is required per the dose modification rules (Section 10.8), the patient will not be dosed. The patient will return every 1-3 weeks 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).
Dosing: The patient dose must be calculated based on the patient's weight on the day of dosing. Dose modification rules and titration rules must be reviewed and implemented prior to dosing as required per protocol (see Section 10.8 and Section 10.9). A dose titration cannot occur until C3D1.

[3] **Medical History:** Medical history for patients with treatment interruption will include medical events occurring after EOS visit in study A536-03 and prior to C1D1 for study A536-05. Medical history for patients without treatment interruption will be taken from study A536-03.

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

[5] **Vital signs** will include weight, heart rate, and systolic and diastolic blood pressure.

[6] **ECG:** May be collected within 28 days prior to C1D1.

[7] **Bone marrow aspirate:** Patients with treatment interruption must have a bone marrow aspirate including cytogenetics performed ≤ 3 months prior to C1D1 for evaluation of eligibility per inclusion criteria Section 9.2.1. A bone marrow aspirate will not be performed on C1D1 for patients without treatment interruption. A bone marrow aspirate must be performed within 21 days after C9D1, C17D1, C25D1, C41D1, C57D1, and C73D1 for all patients. At the EOT visit, a bone marrow aspirate is required for all patients, unless performed < 3 months following a previous bone marrow aspirate and not clinically indicated. Refer to the Laboratory Manual for processing and shipping instructions.

[8] **Serum Iron Studies:** May include serum iron, TIBC, transferrin, soluble transferrin receptor, ferritin.

[9] **Hematology:** RBC, WBC with differential, hemoglobin, hematocrit, nucleated red blood cells (nRBC), reticulocyte count, platelet count, peripheral blasts, 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 may be drawn and resulted (up to 1 day) prior to dosing (see Section 10.8, Individual Dose Modification Rules).

[10] **Serum Chemistry**: Sodium, potassium, AST, ALT, lactate dehydrogenase (LDH), total bilirubin, direct bilirubin, alkaline phosphatase, blood urea nitrogen (BUN)/urea, creatinine, gamma-glutamyl transpeptidase (GGT), calcium, phosphorus, glucose, amylase, lipase, total protein, albumin, and uric acid.

[11] **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.

[12] 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.

[13] **PD Biomarkers:** May include hepcidin, GDF15, GDF8, GDF11, Activin A and others to be determined.

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

[15] **Transfusion Frequency:** Transfusion history will be collected from the EOS visit in the base study A536-03 through the C1D1 visit of A536-05 as available for patients with treatment interruption up to 24 weeks. TD patients will have a defined "pre-transfusion hemoglobin threshold" for requiring transfusion during the study 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 A536-03 (for TD patients without treatment interruption) or study A536-05 (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 should not be transfused if hemoglobin is ≥ 9 g/dL unless indicated for symptoms related to anemia or other reasons at the investigator's discretion.

Cycles 16 through 87: Patients may complete up to 87 Cycles. The last dose of ACE-536 may not be administered after 87 cycles or 1825 calendar days from C1D1, whichever occurs first.

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, followed by PTFU, LTFU, and EOS visits.

Post-Treatment Follow-Up (PTFU): Patients will complete the PTFU visit 2 months (± 7 days) after the last dose of ACE-536

Long-Term Follow-Up (LTFU): Patients will complete LTFU visits every 3 months (± 7 days) after the last dose of ACE-536 for 3 years after the last dose of ACE-536.

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

Long-Term Post-Treatment Follow-Up for overall survival (OS), progression to AML, other malignancies/pre-malignancies (please refer to Section 14.1 for details) may be conducted by record review (including public records if allowed by local regulations) and/or telephone contact with the patient, family, or the patient's treating physician. The investigator must make every effort to obtain information regarding the patient's survival status before determining the patient is lost to follow-up.