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Statistical Analysis Plan

A Phase 2 Randomized, Double-Blind, Placebo-Controlled Study of ACE-083 in Patients with Facioscapulohumeral Muscular Dystrophy

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The undersigned have approved this Statistical Analysis Plan Version 1 for use in this study.

Signature: [redacted] **Date:** 21/Aug/2019
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TABLE OF CONTENTS

1. INTRODUCTION 5
2. STUDY OBJECTIVES 5
2.1. Primary Objective 5
2.2. Secondary Objectives 5
2.3. Exploratory Objectives 6
3. OVERALL STUDY DESIGN 7
3.1. Part 1 7
3.2. Part 2 8
4. STATISTICAL METHODS 9
4.1. General Method 9
4.1.1. Sample Size Determination 9
4.1.2. Computing Environment 9
4.1.3. Treatment Description 9
4.1.4. Definitions of Baseline 10
4.1.5. Data Handling Conventions 11
4.2. Analysis Population 12
4.3. Subject Disposition 13
4.4. Demographic and Baseline Characteristics 14
4.4.1. Demographics 14
4.4.2. Baseline Characteristics 14
4.5. Prior and Concomitant Medications/Therapy 17
4.6. Study Drug Exposure 17
4.6.1. Extent of Exposure 17
4.7. Efficacy Data 18
4.7.1. Variables 18
4.7.1.1. MRI 18
4.7.1.2. Muscle Strenght 19
4.7.1.3. Motor Function 20
4.7.1.4. Patient Reported Outcome Variables 23
4.7.2. Analyses 23
4.7.2.1. Part 1 23

4.7.2.2. Part 2 25
4.7.2.3. Part 2 Open-Label Extension 43
4.7.2.4. Subgroup Analyses (Part 2 Only) 45
4.7.2.5. Other Analyses 47
4.8. Pharmacodynamic Data 48
4.8.1. Variables 48
4.8.2. Analyses 48
4.8.2.1. Part 1 48
4.8.2.2. Part 2 48
4.9. Safety Data 48
4.9.1. Adverse Events 48
4.9.2. Clinical Laboratory Evaluations 50
4.9.3. Anti-Drug Antibodies 51
4.9.4. Other Safety Data 51
4.10. Pharmacokinetic Data 51
5. MODIFICATIONS FROM THE STATISTICAL SECTION OF THE PROTOCOL 52
6. PROGRAMMING SPECIFICATIONS 53
7. APPENDICES 56
APPENDIX 1. SCHEDULE OF EVENTS 57

LIST OF TABLES

Table 1: Planned Doses to Be Administered for Part 1 7

1. INTRODUCTION

This statistical analysis plan (SAP) provides a comprehensive and detailed description of strategy and statistical techniques to be used to analyze data for study protocol A083-02. The purpose of the SAP is to ensure the credibility of the study findings by pre-specifying the statistical approaches to the analysis of study data prior to database lock.

2. STUDY OBJECTIVES

2.1. Primary Objective

Part 1

- To evaluate the safety and tolerability of ACE-083 in patients with facioscapulohumeral disease (FSHD)

Part 2

- To determine whether treatment with ACE-083 increases total muscle volume of the injected muscle in patients with FSHD

2.2. Secondary Objectives

Part 1

- To determine the recommended dose level(s) of ACE-083 for Part 2
- To evaluate changes in muscle volume and intramuscular fat fraction of the injected muscle
- To evaluate changes in strength of the injected muscle
- To estimate the systemic exposure of ACE-083 when administered as a local muscle injection

Part 2

- To determine whether treatment with ACE-083 decreases intramuscular fat fraction of the injected muscle
- To determine whether treatment with ACE-083 increases strength of the injected muscle
- To determine whether treatment with ACE-083 improves motor function related to the injected muscle
- To evaluate changes in patient-reported measures of FSHD-HI total score and subscale scores
- To evaluate safety and tolerability of ACE-083
- To estimate the systemic exposure of ACE-083 when administered as a local muscle injection

2.3. Exploratory Objectives

Part 1

- To evaluate changes in motor function related to the injected muscle
- To evaluate changes in patient-reported measures of FSHD-HI total score and subscale scores.

3. OVERALL STUDY DESIGN

This study is a two-part multicenter, phase 2 study to evaluate the safety, tolerability, pharmacodynamics (PD), efficacy, and pharmacokinetics (PK) of ACE-083 in patients with FSHD. Part 1 is open-label dose-escalation and Part 2 is randomized, double blind, placebo-controlled with an open-label extension.

Patients who participate in Part 1 are not eligible to participate in Part 2.

The total study duration for individual patients in Part 1 will be approximately 6 months, which includes a 1-month screening period, a 3-month treatment period, and a 2-month follow-up period after the last dose. The total study duration for individual patients in Part 2 will be approximately 15 months, which includes a 1-month screening period, a 12-month treatment period (6-month double-blind, placebo-controlled followed by a 6-month open-label extension), and a 2-month follow-up period after the last dose.

3.1. Part 1

Part 1 will consist of up to 6 cohorts of patients and will evaluate multiple ascending dose levels of ACE-083 in either the tibialis anterior (TA) or biceps brachii (BB) muscle. Patients in each cohort will be enrolled in a 1-month screening period before beginning treatment.

Table 1 below outlines the planned doses to be administered.

6-month intervals thereafter.

The primary endpoint analysis will be performed when all participants have completed their post-Treatment Period PVR assessment.

A detailed charter will outline all activities of the DMC (including, but not limited to, type of data to be reviewed, DMC responsibilities, and frequency of meetings).

Table 1: Planned Doses to Be Administered for Part 1

Cohort	Dosing Days	Muscle	Planned Dose Level	Type of Administration	ACE-083 n
1a	1, 22, 43, 64, and 85	Tibialis Anterior	150 mg	Unilateral	6
2a	1, 22, 43, 64, and 85	Tibialis Anterior	200 mg	Unilateral	6
3a	1, 22, 43, 64, and 85	Tibialis Anterior	200 mg	Bilateral	6
Total Number of Patients (Planned)					**18**

Cohort	Dosing Days	Muscle	Planned Dose Level	Type of Administration	ACE-083 n
1b	1, 22, 43, 64, and 85	Biceps brachii	150 mg	Unilateral	6
2b	1, 22, 43, 64, and 85	Biceps brachii	200 mg	Unilateral	6
3b	1, 22, 43, 64, and 85	Biceps brachii	240 mg	Unilateral	6
Total Number of Patients (Planned)					**18**

Periodic reviews of selected blinded safety and imaging data will be reviewed by the Safety Review Team (SRT) in accordance with the SRT Guidelines.

3.2. Part 2

Prior to the initiation of Part 2, a review of the safety and efficacy data from Part 1 will be done in order to determine whether one or both the TA and BB muscles will be studied as well as the recommended dose level for each muscle. Up to 56 patients (28 patients per muscle) will be enrolled and randomized (1:1 ratio) to receive either ACE-083 (n=14/muscle) or placebo (n=14/muscle) bilaterally to either the TA or BB muscles (but not both). Patients will receive study drug once every three weeks for approximately 6 months (9 doses). If a patient discontinues the study for reasons other than a safety issue related to investigational study drug and the patient had not completed the Day 43 visit, the patient may be replaced. The replacement patient will receive the same treatment as the patient replaced.

Patients who complete the double-blind treatment period will immediately roll over to open-label treatment with ACE-083. All patients will receive ACE-083 administered at the same dose as in the double-blind period. They will receive bilateral injections, in the same muscles that study drug was administered to during the double-blind period, once every three weeks for approximately 6 months (8 doses). In Part 2, the SRT will periodically review blinded safety data for each muscle treated.

A stratified randomization schedule will be prepared under the direction of the Sponsor with the stratification factors of: “Muscle” (values are “Tibialis anterior (TA)” and “Biceps brachii (BB)”) and “MRC-MMT Grade” (values are “3 to 4-” and “4- to 4+”). Stratification by baseline MRC-MMT grade will be that of the weaker side. Patients whose weaker MRC-MMT grade is “4-“ will be randomly assigned to either the “3 to 4-“ or the “4- to 4+” stratum based on the Interactive Response Technology (IRT) system. Because Part 2 is multicenter, an IRT system will be used to randomize patients sequentially across clinical sites.

Similar to Part 1, periodic reviews of selected blinded safety and imaging data will be reviewed. Further details concerning the frequency and timing of SRT reviews of Part 2 data are described in the SRT Guidelines.

4. STATISTICAL METHODS

4.1. General Method

The Full Analysis Set consists of all randomized participants treated with correct treatment assignment.

4.1.1. Sample Size Determination

There was no formal sample size calculation for Part 1. Six patients in each cohort will provide sufficient data to evaluate safety and a preliminary assessment of changes in muscle volume and muscle strength.

The sample size calculation for Part 2 is based upon the percent change from baseline in total muscle volume of the injected TA muscle 3 weeks after the last dose. A 10% difference between ACE-083-treated and placebo groups in the percent change in total muscle volume from baseline is considered to be clinically meaningful. The standard deviation (SD) is assumed to be approximately 9% for each group, based on preliminary MRI data for the ACE-083-treated side from the initial TA cohort in Part 1. Similar assumptions apply for the treatment of the BB muscle.

Assuming a 2-sided type I error rate of 0.10, a 10% difference in percent change from baseline between ACE-083-treated and placebo groups in total muscle volume, a standard deviation of 9% for each group, and a 1:1 randomization, 83% power is achieved with a total sample size of 24 for the TA muscle (12 active, 12 placebo), based on a standard t-test.

In addition, this sample size also provides 83% power to detect a 10% difference in 6-minute walk test distance, based on a similar estimated SD of 9% and a 2-sided type 1 error rate of 0.10.

In order to account for dropouts (up to 15%), 28 patients will be randomized to study treatment for each muscle (14 active, 14 placebo) to ensure that at least 12 patients per treatment group complete the double-blind treatment period.

4.1.2. Computing Environment

The analysis of clinical data from both Parts 1 and 2 will be performed under the direction of the Acceleron Pharma Biostatistics Department, using SAS® (version 9.4 or higher) and R.

4.1.3. Treatment Description

Treatment descriptions as well as abbreviations used in tables, listings, and figures are described below:

Part 1

Unless otherwise stated, tables and figures will be presented as shown below:

Part 1 – Tibialis Anterior

ACE-083 150 mg Unilateral	ACE-083 200 mg Unilateral	ACE-083 200 mg Bilateral	Overall

Part 1 – Biceps Brachii

ACE-083 150 mg Unilateral	ACE-083 200 mg Unilateral	ACE-083 240 mg Unilateral	Overall

Listings will contain only the treatment groups shown with no "Overall" group.

Part 2

Unless otherwise stated, tables and figures will be presented using one or more of the following as shown below, depending on what is being summarized/analyzed.

Part 2 – Double-blind period

Tibialis Anterior			Biceps Brachii		
Placebo	ACE-083	Overall	Placebo	ACE-083	Overall

Part 2 – Open-label period –by treatment group sequence

Tibialis Anterior		Biceps Brachii	
ACE-083→ACE-083	Placebo→ACE-083	ACE-083→ACE-083	Placebo→ACE-083

Part 2 – Open-label period – Pooled across treatment group sequence

Tibialis Anterior	Biceps Brachii
ACE-083	ACE-083

Listings will contain the treatment groups shown above however, there will be no "Overall" group.

4.1.4. Definitions of Baseline

Part 1 and Part 2 (Double-blind phase)

For MRI, PD, and safety data, baseline is defined as the last non-missing assessment prior to the first dose of study drug administration. Theoretically, this is the Day 1 pre-dose assessment. For patients that are randomized (applicable to patients participating in Part 2) but do not receive any study drug administration, the date of randomization will be the baseline reference.

For motor function data (lower leg and upper arm functional assessments), strength (QMT by handheld device), gait, and FSHD-HI data whose pre-treatment assessments are performed up to three times prior to the first dose, the baseline is defined as the average of the non-missing values. Theoretically, this would be at the Screening Day -28, Screening Day -7, and Day 1 visits. The first dose date will serve as the reference from which the non-missing pre-treatment values would be identified. For patients that are randomized (applicable to patients participating in Part 2) but do not receive any study drug administration, the date of randomization will be the baseline reference. The last non-missing assessment prior to the first dose of study drug administration will also be determined for this data and will be available for sensitivity analyses for any analyses of changes from baseline as appropriate.

Part 2 (Open-label extension)

For assessments performed in the open-label extension where data prior to the start of the open-label extension is collected at both Day 169 and 190, the baseline is defined as the mean of the assessments performed at Day 169 and Day 190. Should one of these assessments be missing, the other assessment would be used as the baseline.

For assessments performed in the open-label extension where data prior to the open-label extension is only collected at Day 190 (e.g. MRI, FSHD-HI, strength), then the baseline would be the Day 190 assessment.

4.1.5. Data Handling Conventions

Unless otherwise stated or indicated:

- Individual data for all patients will be presented in data listings, sorted by "Study Part" (Part 1 or Part 2), "Treatment Group", and "Patient Number";
- For any parameter analyzed, only observations from visits and/or time points planned in the protocol will be used in descriptive statistics. An exception to this rule is for a baseline assessment. Should an unscheduled assessment be taken after the scheduled baseline assessment but before the first dose of study medication, then the unscheduled assessment may be used as the baseline;
- For variables where the Day 1 assessment is defined as the baseline, should an individual patient have a Day 1 assessment after having received their first dose, then an unscheduled assessment may be used as the baseline, provided that the unscheduled assessment was done prior to first dose.
- For variables where the baseline is defined as the mean of Day -28, Day -7, and Day 1, should an individual patient have a Day 1 assessment taken after having received their first dose, then only the Day -28, Day -7, and any unscheduled assessment will be used to determine the average for the baseline (as described in Section 4.1.4), provided that the unscheduled assessment was done prior to first dose.
- For the determination of baseline PUL, only data on the patient's preferred side will be used. For example, if the patient's preferred side is "Left" and any individual PUL assessment at any of the Day -28, Day -7, and Day 1 timepoints is recorded as having been done on the "Right" or performed as "Both", that individual assessment will not be used in the determination of the baseline PUL for that patient.
- With the exception of what is described in the preceeding bullet, unscheduled assessements will not be collectively summarized in summary tables or figures; however, these data will appear in individual patient listings.
- For adverse events where the assessment of "Relationship to study drug" is missing, it will be assumed that the adverse event is "Probably Related" to study drug.
- For selected efficacy data, tests of statistical hypotheses and estimation using the end-of-treatment visit or the Day 190 visit (Part 2 double-blind component), multiple imputation for missing data will be performed. There will be no imputations performed for patients who discontinued prior to Day 43 and were replaced.

Additional techniques for handling missing data may also be considered and evaluated.

- If a patient has a Day 190 assessment performed after having received the Day 190 dose (first dose in the open-label phase), then the Day 190 assessment for that patient will be included in statistical analyses of Day 190 data only if the difference between the Day 190 assessment and the Day 190 dose date is less than or equal to 7 days.
- Descriptive statistics for quantitative parameters will be provided using number of observations (N), mean, standard deviation (SD), standard error of the mean (SEM), median, minimum and maximum;
- Should it be necessary to convert duration in days to duration in months, the duration in days will be divided by 30.417 (365/12) to calculate duration in months.
- Descriptive statistics for qualitative parameters will be provided using frequencies (N) and percentages (%).
- If the result of a continuous variable contains nonnumeric values (e.g. '<X' or '>X'), the imputed values used for the descriptive statistics will be determined by considering the following rules:
  - If the value is '<X', the value used will be X/2;
  - If the value is '>X', the value used will be X.
- Concerning the determination of absolute and percent change from baseline for data such as MRI and muscle strength where assessments are reported separately for the left and right sides and the average absolute and/or percent change from baseline is needed, the absolute and/or percent change is determined for the left and right sides separately and then averaged. The "average" represents the "average" absolute (or percent) change from baseline for the left and right sides. For MRI and muscle strength data, should a patient have an MRI and/or strength assessment on one side (i.e. right side) but not the other side at an individual timepoint, the value for the average absolute (or percent) change from baseline for that patient and timepoint will be the absolute (or percent) change from the side from which the MRI and/or strength assessment was obtained.

4.2. Analysis Population

The analysis populations include the following:

- Full Analysis Set: Part 1 – All patients enrolled in the study receiving at least one dose of ACE-083. Part 2 – All patients randomized;
- Safety Set: All patients enrolled/randomized in the study who have received at least one dose of study drug (includes placebo);
- Per Protocol Set: All patients enrolled/randomized in the study who have received at least one dose of study drug (includes placebo) with no major protocol violations.
  - Major protocol violations include:

- Patients who discontinue the study prior to the Cycle 3 Day 43 visit; these patients are replaced per protocol
- Patients whose Day 1 MRI or Day 190 MRI was done 7 or more days following study drug administration
- Patients whose functional assessments (i.e. 10mW/R, mid-level PUL, 6MWT, 4-stair climb) at Day 1 or Day 190 were done 7 or more days following study drug administration
- Patients who did not receive the full study drug administration planned per protocol at all injection sites on any or both sides (right or left) for 2 or more consecutive visits for reasons other than dose reductions for the safety of the patient or feasibility concern (i.e., the site intended to administer the full dose but was unable).

Should the statistical analysis populations be identical (e.g., Full Analysis Set = Per Protocol Set), statistical analyses will not be duplicated or repeated.

4.3. Subject Disposition

Individual patient disposition data will be listed.

Frequency counts will be tabulated for disposition data and will include the following:

- Number of patients enrolled [Part 1 only],
- Number of patients randomized [Part 2 only],
- Number (%) of patients completing the study [Part 1 only],
- Number (%) of patients completing the double-blind treatment period [Part 2 only]
  - A patient is said to have completed the double-blind treatment period if the patient completed up to and including the Day 190 visit.
- Number (%) of patients entered into the open-label period [Part 2 only]
- Number (%) of patients completing the open-label treatment period [Part 2 only]
  - A patient is said to have completed the open-label treatment period if the patient completed up to and including the Day 337 visit.
- Number (%) of patients that rollover to the extension study, A083-04
  - Patients that rollover to the extension study consist only of patients participating in Part 2 of this study who complete both the double-blind treatment period AND the open-label treatment period who will not have the Day 393/EOS visit. These patients' final study visit will be the Day 358/ET visit.
- Number (%) of patients that do not rollover to the extension study A083-04
  - Patients that do not rollover to the extension study will have both the Day 358/ET and Day 393/EOS visits completed.

- Number (%) of patients discontuing from the study prior to completing both the double-blind period and the open label period [Part 2 only]
  - Number (%) of patients that discontinued during the double-blind period
  - Number (%) of patients that discontinued during the open-label period
  - Number (%) of patients for primary reason for discontinuation will also be provided [provided there is at least one patient who discontinued].

Summaries will be provided by treatment and overall for each study part (Part 1 and Part 2).

4.4. Demographic and Baseline Characteristics

4.4.1. Demographics

Individual demographic data will be listed.

Descriptive statistics (N, mean, SD, median, minimum and maximum) will be provided for continuous demographic variables (age, weight, height, and BMI) and frequency counts will be tabulated for categorical demographic variables (gender, race, ethnicity) by treatment and overall for each study part (Part 1 and Part 2). Age will be calculated based on birth date and informed consent date.

4.4.2. Baseline Characteristics

Baseline characteristics of the patient population consist of selected items from the FSHD disease history, MRI, MRC-MMT, and functional test data. Such data includes the following:

- FSHD disease diagnosis
  - The disease diagnosis values are either FSHD1 or FSHD2
- D4Z4 fragment size (kilobases)
  - The D4Z4 fragment sizes will be grouped into the following categories: $\leq$ 18 kb; 19-28 kb; > 28 kb (These categories roughly correspond with categories based on number of D4Z4 repeats.)
- Scapular fixation surgery
  - This is a categorical variable whose possible values are “Right”, “Left”, “Both”, or “No History”.
  - This is derived off of the responses to the question “Did the patient have history of scapular fixation surgery” (responses are “Yes” or “No”) and if “Yes”, then the choice of either “Right”, “Left”, or “Both” would be selected.
  - A value of “No History” would be the case where the answer to the question “Did the patient have history of scapular fixation surgery” is “No”.
- Wears any lower limb orthotics/braces
  - This is a categorical variable whose values are “Right”, “Left”, “Both”, or “Does not wear”.

  - This is derived off of the responses to the question “Does the patient currently wear any lower limb orthotics/braces” (responses are “Yes” or “No”) and if “Yes”, then the choice of either “Right”, “Left”, or “Both” would be selected.
  - A value of “Does not wear” would be the case where the answer to the question “Does the patient currently wear any lower limb orthotics/braces?” is “No”.
- Walking device use
  - Values are “Yes” or “No”
- First degree relatives with FSHD
  - Values are “Yes” or “No”
- Exercise program
  - This is a categorical variable whose values are “Aerobic 1-2 times per week”, “Aerobic 3-4 times per week”, “Aerobic 5+ times per week”, “Resistance training 1-2 times per week”, “Resistance training 3-4 times per week”, “Resistance training 5+ times per week”, “Both aerobic and resistance training 1-2 times per week”, “Both aerobic and resistance training 3-4 times per week”, “Both aerobic and resistance training 5+ times per week”, and “No exercise program”
  - This is derived off of the responses to the question “Does the patient have a regular exercise program” (responses are “Yes” or “No”) and if “Yes”, then the choices of how many times a week overall (1-2, 3-4, or 5+) and the type of exercise (Aerobic, Resistance training, or Both) would be selected.
- Age at onset of symptoms (years)
- Age at diagnosis (years)
- Duration since onset of symptoms (years)
  - Duration since onset of symptoms = Age (years) – Age at onset of symptoms (years)
- Duration since diagnosis (years)
  - Duration since diagnosis = Age (years) – Age at diagnosis (years)
- Total muscle volume (mm3)
  - See Section 4.7.1 for definition.
- Total muscle mass (g)
  - See Section 4.7.1 for definition.
- Calculated contractile muscle volume (mm3)
  - See Section 4.7.1 for definition.
- Calculated contractile muscle mass (g)
  - See Section 4.7.1 for definition.

- Fat fraction (%)
- 6-minute walk distance (m)
- Time to complete 10 meter walk/run (s)
- PUL composite time (s)
- MRC-MMT grade category

Part 1

- MRC-MMT grades will be grouped into the following categories: [3 to 4-], [4 to 4+], [5- to 5]
- For unilateral dose cohorts, summaries to be provided will be the side treated and will either be that of elbow flexion (for those receiving study drug in the BB muscle), ankle dorsiflexion (for those receiving study drug in the TA muscle), or knee extension (for those receiving study drug in the TA muscle).
- For those receiving study drug bilaterally, each side will be summarized
  - Elbow flexion (for those receiving study drug in the BB muscle)
  - Ankle dorsiflexion (for those receiving study drug in the TA muscle)
  - Knee extension (for those receiving study drug in the TA muscle).

Part 2

- MRC-MMT grade classification: [3 to 4-] and [4 to 4+].
- MRC-MMT grades will also be summarized (frequency with percents) by the weakest grade for each of the following:
- Elbow flexion (BB patients) – possible values are 3, 3+, 4-, 4, 4+
- Ankle dorsiflexion (TA patients) – possible values are 3, 3+, 4-, 4, 4+
- Knee extension (TA patients) – possible values are 2-, 2, 2+, 3-, 3, 3+, 4-, 4, 4+, 5-, 5.
- MRC-MMT SCORE which represents the conversion of the MRC-MMT grade to the decimal version as follows: 5 = 5.0, 5- = 4.67, 4+ = 4.33, 4 = 4.0, 4- = 3.67, 3+ = 3.33, 3 = 3.0, 3- = 2.67, 2+ = 2.33, 2 = 2.0, 2- = 1.67, 1+ = 1.33, 1 = 1.0.
  - For unilateral dose cohorts, summaries to be provided will be for the side treated and will either be that of elbow flexion (for those receiving study drug in the BB muscle) or ankle dorsiflexion (for those receiving study drug in the TA muscle).
  - For bilateral dose cohorts, this will be determined for each side (left and right) and then averaged. Only the "average" will be presented in the summary table.
- Dose of ACE-083 expressed as (mg/g calculated total muscle mass)
- Dose of ACE-083 expressed as (mg/g calculated contractile muscle mass)

The table below outlines how to derive the dose of ACE-083 expressed as either mg/g total muscle, or mg/g contractile muscle:

	Dose of ACE-083 Expressed as	
	mg/g Calculated Total Muscle Mass	**mg/g Calculated Contractile Muscle Mass**
Unilateral Cohorts	Dose injected (mg) / (Baseline CCMV {mm3}* [1.06/1000] + Baseline CIMFV {mm3} * [0.9/1000])	Dose injected (mg) / (Baseline CCMV {mm3}* [1.06/1000])
Bilateral Cohorts	Step 1: For each side (right and left), compute the following: Dose injected (mg) /(Baseline CCMV {mm3}* [1.06/1000] + Baseline CIMFV {mm3}* [0.9/1000]) Step 2: Compute average of the Dose of ACE-083 expressed as mg/g total muscle for each side using the information obtained from Step 1.	Step 1: For each side (right and left), compute the following: Dose injected (mg) /(Baseline CCMV {mm3}* [1.06/1000]) Step 2: Compute average of the Dose of ACE-083 expressed as mg/g contractile muscle for each side using the information obtained from Step 1.

CCMV = Calculated contractile muscle volume; CIMFV = Calculated intramuscular fat volume
Note: For unilateral cohorts, this will be determined only for the side that is treated.

This data will be summarized using appropriate descriptive statistics.

4.5. Prior and Concomitant Medications/Therapy

Prior and concomitant medications recorded during the study will be listed. These will be coded with the WHO Drug Dictionary (Version 2016-09).

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 classified as a prior medication.
- If the start date is on or after the first dose date of study drug, the medication will be classified as a concomitant medication.
- 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 classified 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 classified 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 classified as concomitant.
- If the start and stop dates are missing, the medication will be classified as concomitant.

4.6. Study Drug Exposure

4.6.1. Extent of Exposure

A listing of individual patient dosing data will be provided.

Study drug exposure will be summarized descriptively by cohort (Part 1) and by treatment group for each muscle (Part 2) for the safety population. Such summaries will include the total number

of treatment cycles, the number and percentage of patients with dose delay and reduction. If no patients experience dose delay and reduction, then no such summaries will be provided.

The total number of cycles will be summarized by presenting the number and percentage of patients in each category. Categories refer to the total number of treatment cycles. For Part 1, the possible values of the total number of treatment cycles are: "1", "2", "3", "4", and "5". For Part 2 (double-blind period), the possible values of the total number of treatment cycles over the course of the double-blind treatment period is: "1", "2", "3", "4", "5", "6", "7", "8", or "9". For Part 2 (open-label extension), the possible values of the total number of treatment cycles over the course of the open-label extension is: "1", "2", "3", "4", "5", "6", "7", or "8".

The total study drug exposure in weeks will also be summarized and will be derived according to the following formula: Exposure (weeks) = [(Last dose date – First dose date) +21] / 7.

For Part 1, summaries of the total study drug exposure in weeks will be provided by cohort using descriptive statistics. The summary table will include a pooled summary across cohorts within each muscle treated.

For Part 2, summaries of the total study drug exposure in weeks will be provided by treatment group for each muscle treated.

4.7. Efficacy Data

4.7.1. Variables

4.7.1.1. MRI

MRI variables consist of the following:

Variable	Unit	Formula for derivation or SAS variable name/expression from SDTM FA
Total muscle volume (TMV)	mm^3	TMV = Contractile muscle tissue volume (SAS variable FASTRESN in SDTM FA where FATESTCD = "MVOL") + Intramuscular fat volume (SAS variable FASTRESN in SDTM FA where FATESTCD="IFATVOL")
Intramuscular fat volume (IMFV)	mm^3	SAS variable FASTRESN in SDTM FA where FATESTCD="IFATVOL"
Contractile muscle volume (CMV)	mm^3	SAS variable FASTRESN in SDTM FA where FATESTCD="MVOL"
Fat fraction (FF)	%	SAS variable FASTRESN in SDTM FA where FATESTCD="FATF"
Contractile muscle fraction (CMF)	%	CMF = 100 – FF
Calculated contractile muscle volume (CCMV)	mm^3	CCMV = TMV * CMF/100
Calculated intramuscular fat volume (CIMFV)	mm^3	CIMFV = TMV * FF/100
Contractile muscle mass (CMM)	g	CMM = CMV * 1.06/1000

Variable	Unit	Formula for derivation or SAS variable name/expression from SDTM FA
Intramuscular fat mass (IMFM)	g	IMFM = IMFV * 0.9/1000
Total muscle mass (TMM)	g	TMM = CMM + IMFM
Calculated contractile muscle mass (CCMM)	g	CCMM = CCMV * 1.06/1000
Calculated intramuscular fat mass (CIMFM)	g	CIMFM = CIMFV * 0.9/1000
Calculated total muscle mass (CTMM)	g	CTMM = CCMM + CIMFM

4.7.1.2. Muscle Strength

Muscle strength variables consist of the following:

- For cohorts where treatment is administered unilaterally
  - Ankle dorsiflexion (maximum voluntary isometric contraction [MVIC]) for the treated TA muscle [TA cohorts]
    a. MVIC = maximum force (from 3 measurements) from the hand-held dynamometer.
    b. The treated TA muscle can be either the right or left side. The treated and untreated sides will remain the same for the individual patient throughout the study.
  - Elbow flexion (MVIC) for the treated BB muscle [BB cohorts]
    a. MVIC = maximum force (from 3 measurements) from the hand-held dynamometer.
    b. The treated BB muscle can be either the left or right side. The treated and untreated sides will remain the same for the individual patient throughout the study.
- For cohorts where treatment is administered bilaterally
  - Maximum (of 3 measurements) right ankle dorsiflexion (MVIC) [TA cohorts]
  - Maximum (of 3 measurements) left ankle dorsiflexion (MVIC) [TA cohorts]
  - Maximum (of 3 measurements) right elbow flexion (MVIC) [BB cohorts]
  - Maximum (of 3 measurements) left elbow flexion (MVIC) [BB cohorts]

For each of the MRI and muscle strength variables listed above, the absolute and percent change from baseline will be determined for each side ("Treated", "Untreated", and "Treated – Untreated" sides for unilateral dose cohorts; "Left", and "Right" sides for bilateral dose cohorts) and time. In addition, for bilateral dose cohorts, the average of the absolute change from baseline as well as the average of the percent change from baseline for the left and right sides will also be determined.

4.7.1.3. Motor Function

4.7.1.3.1. Lower Leg Function Assessments

10-Meter Walk/Run Test

- Time to complete 10-meter walk/run (seconds)

4-Stair Climb

- Ascending
  - Time to ascending stairs (sec.)
  - Number and percentage of patients belonging to the following grades:

1. Unable to climb 4 standard stairs
2. Climbs 4 standard stairs “marking time” (climbs one foot at a time, with both feet on a step before moving to next step), uses both arms on one or both handrails
3. Climbs 4 standard stairs “marking time” (climbs one foot at a time, with both feet on a step before moving to next step), using one arm on one handrail
4. Climbs 4 standard stairs “marking time” (climbs one foot at a time, with both feet on a step before moving to next step), not needing handrail
5. Climbs 4 standard stairs alternating feet, needs handrail for support
6. Climbs 4 standard stairs alternating feet, not needing handrail support
7. Not Done

- Descending
  - Time to descending stairs (sec.)
  - Number and percentage of patients belonging to the following grades:

1. Unable to descend 4 standard stairs
2. Descends 4 standard stairs “marking time” (climbs one foot at a time, with both feet on a step before moving to next step), uses both arms on one or both handrails
3. Descends 4 standard stairs “marking time” (climbs one foot at a time, with both feet on a step before moving to next step), using one arm on one handrail
4. Descends 4 standard stairs “marking time” (climbs one foot at a time, with both feet on a step before moving to next step), not needing handrail
5. Descends 4 standard stairs alternating feet, needs handrail for support
6. Descends 4 standard stairs alternating feet, not needing handrail support
7. Not Done

- **6-Minute Walk Test**
  - Cumulative distance traveled at 6 minutes (meters)
  - Cumulative distance traveled at 1 minute (meters)
  - Cumulative distance traveled at 2 minutes (meters)
  - Cumulative distance traveled at 3 minutes (meters)
  - Cumulative distance traveled at 4 minutes (meters)
  - Cumulative distance traveled at 5 minutes (meters)
- Gait Assessments
  - General measures: Ambulation distance (meters), Ambulation time (ms), Ambulation velocity (cm/s), Mean normalized velocity (Leg lengths/s), Number of steps, Cadence (steps/min.), Step time differential (ms), Step length differential (m), Cycle time differential (ms), Forward lean (deg.), Sway (m), Left step time (ms), Left Cycle Time (ms), Left Step Length (m), Left stride length (m), Left base width (m), Left single support (% gait cycle), Left initial double support (% gait cycle), Left terminal double support (% gait cycle), Left total double support (% gait cycle), Left swing (% gait cycle), Left stance (% gait cycle), Left step extremity ratio (ratio), Right step time (ms), Right Cycle Time (ms), Right Step Length (m), Right stride length (m), Right base width (m), Right single support (% gait cycle), Right initial double support (% gait cycle), Right terminal double support (% gait cycle), Right total double support (% gait cycle), Right swing (% gait cycle), Right stance (% gait cycle), Right step extremity ratio (ratio)
  - Initial measures: Initial left hip flexion (deg.), initial left knee flexion (deg.), initial left ankle dorsiflexion (deg.), initial left clearance (cm), Initial right hip flexion (deg.), initial right knee flexion (deg.), initial right ankle dorsiflexion (deg.), initial right clearance (cm)
  - Peak measures: Peak left hip min (deg.), Peak left hip max (deg.), Peak left knee min (deg.), Peak left knee max (deg.), Peak left ankle dorsiflexion min (deg.), Peak left ankle dorsiflexion max (deg.), Peak left clearance max (cm.), Peak right hip min (deg.), Peak right hip max (deg.), Peak right knee min (deg.), Peak right knee max (deg.), Peak right ankle dorsiflexion min (deg.), Peak right ankle dorsiflexion max (deg.), Peak right clearance max (cm.)
  - Average measures: Average left hip min (deg.), Average left hip max (deg.), Average left knee min (deg.), Average left knee max (deg.), Average left ankle dorsiflexion min (deg.), Average left ankle dorsiflexion max (deg.), Average left clearance max (cm.), Average right hip min (deg.), Average right hip max (deg.), Average right knee min (deg.), Average right knee max (deg.), Average right ankle dorsiflexion min (deg.), Average right ankle dorsiflexion max (deg.), Average right clearance max (cm.)
- 100-meter timed test (patients receiving ACE-083 in the TA muscle during the Part 2 open-label phase)

- Time to complete 100-meters (sec.)

4.7.1.3.2. Upper Arm Functional Assessments

- Performance of upper limb (PUL) – Middle level elbow dimension score
  - Sum of the 9 items for this particular dimension (Maximum = 34). These include: "Hand(s) to Mouth", "Hand(s) to table from lap", "Move weight on table", "Lifting light cans", "Lifting heavy cans", "Stacking light cans", "Stacking heavy cans", "Remove lid from container", and "Tearing paper".
  - Time to lift 5 light cans (sec.)
  - Time to stack 5 light cans (sec.)
  - Time to lift 5 heavy cans (sec.)
  - Time to stack 5 heavy cans (sec.)
  - Composite (or average) time to complete the following tasks: lift 5 light cans, lift 5 heavy cans, stack 5 light cans, and stack 5 heavy cans.

a. Note that when deriving the average time to complete the selected tasks listed above, one or more of the times may be missing for a given patient at one or more time points. If such a scenario occurs, the average of the "non-missing" times should then be determined. For example, if the "Time to lift 5 light cans (sec.)" is missing for a patient at a particular time point (s), and the other 3 quantities are non-missing, then the average time to complete the selected tasks should be computed off of the 3 non-missing quantities.

- PUL high level/shoulder dimension (patients receiving ACE-083 in the BB muscle during the Part 2 open-label phase)
  - Shoulder domain score which represents the sum of the following 4 items that the patient is asked to do on the preferred side [either right or left side identified by patient to be done on the same side for all scheduled times]:
    a. Largest weight patient can use to perform shoulder abduction to shoulder height (elbow to shoulder level)
    b. Largest weight patient can use to perform shoulder abduction above shoulder height (elbow to eye level)
    c. Largest weight patient can use to perform shoulder flexion to shoulder height (elbow to shoulder level)
    d. Largest weight patient can use to perform shoulder flexion above shoulder height (elbow to eye level)

Each of the 4 components above is scored depending on the highest weight performed where scores are: 0 = Unable; 1 = Able no weights; 2 = 200 g; 3 = 500 g; 4 = 1000 g. The maximum score is 16.

4.7.1.4. Patient Reported Outcome Variables

- Total Facioscapulohumeral Disease Health Index (FSHD-HI) score
- The 14 subscale scores of the FSHD-HI survey
  - Shoulder and Arm Function, Mobility and Ambulation, Fatigue, Cognitive Function, Activity Limitation, Core Strength and Function, Gastrointestinal Function, Social Performance, Body Image, Hand and Finger Function, Social Satisfaction, Pain, Emotional Health, and Communication

4.7.2. Analyses

4.7.2.1. Part 1

Unless otherwise specified, no imputations for missing data will be performed for Part 1 data.

MRI and Muscle Strength Variables

Unilateral Cohorts

Individual MRI and strength data will be listed.

For each MRI and strength variable, the raw data as well as the absolute and percent change from baseline in the treated TA or BB muscle, untreated TA or BB muscle, and the treated minus untreated TA or BB muscle will be summarized for each scheduled time point.

Least square mean estimates of the effect of ACE-083 and corresponding 90% and 95% confidence intervals will be produced using an analysis of covariance (ANCOVA) for the following variables: TMV percent change from baseline 3 weeks post last dose, CCMV percent change from baseline 3 weeks post last dose, FF absolute change from baseline 3 weeks post last dose, percent change in elbow flexion MVIC 3 weeks post last dose (BB cohorts), and percent change in ankle dorsiflexion MVIC 3 weeks post last dose (TA cohorts).

The ANCOVA model will contain the following covariates:

- COHORT represents the dose level of ACE-083 administered to the muscle.
- BASELINE represents the baseline value of the parameter of interest
- BASELINE*COHORT represents the interaction of the baseline and cohort

The analysis described above will be performed for the treated side as well as for the difference in treated and untreated sides and will be done separately for the TA and BB cohorts.

For BB patients, the above analyses will be repeated using the patient's "preferred" side used for the PUL test for patients.

Additional analyses may be performed as appropriate.

Bilateral Cohort

For each MRI and strength parameter, descriptive statistics of the raw data as well as the absolute and percent change from baseline will be provided by side treated [left and right] as well as for the average of the left and right sides.

A one-sample t-test will be performed to estimate the effect of ACE-083 with corresponding 90% and 95% confidence intervals for the following variables: TMV percent change from baseline 3 weeks post last dose, CCMV percent change from baseline 3 weeks post last dose, FF absolute change from baseline 3 weeks post last dose, and percent change in ankle dorsiflexion MVIC 3 weeks post last dose.

The analysis described above will be performed using the data for the average absolute as well as the average percent change from baseline for the left and right sides.

Motor Function Variables

Individual motor function data will be listed.

For motor function test variables (TA cohorts: cumulative distance at 6 minutes from the 6-minute walk test, time to complete 10 meter walk/run test, 4-stair ascend time, 4-stair descend time, gait parameters; BB cohorts: PUL parameters) the raw data as well as absolute and percent change from baseline will be provided.

Least square mean estimates of the effect of ACE-083 and corresponding 90% and 95% confidence intervals will be produced for the percent change data at 3 weeks post last dose described above using an ANCOVA with BASELINE, COHORT, and BASELINE* COHORT as covariates where:

- BASELINE represents the baseline value of the parameter of interest
- COHORT represents the dose level of ACE-083 administered to the muscle
- BASELINE*COHORT represents the interaction of BASELINE and COHORT

Additional analyses may be performed as appropriate.

Patient reported outcome variables

Individual FSHD-HI scored data (total score and each of the subscale scores) will be listed.

Descriptive statistics of the raw data as well as for the absolute and percent change from baseline will be provided for the FSHD-HI total score as well as for each of the subscale scores.

Least square mean estimates of the effect of ACE-083 and corresponding 90% and 95% confidence intervals will be produced for the absolute change data at 3 weeks post last dose described above using an ANCOVA with BASELINE, COHORT and BASELINE*COHORT as covariates where BASELINE, and COHORT are defined similarly as described above for other analyses.

Additional analyses may be performed as appropriate.

Gait

Individual data will be listed.

Descriptive statistics will be provided for each type of measurement recorded (i.e. “General measures”, “Initial measures”, “Peak measures”, and “Average measures”) by cohort and scheduled time.

For gait assessments that are reported in triplicates at a given visit for any patient and are assessed to be of good quality, the mean of these will be used for purposes of generating a single individual value for the patient at the scheduled visit which will then be summarized.

In addition, "Stride Length" will be derived using the following formula:

> Stride Length (m) = {Mean of the triplicate measures of Left Step Length (m)} + {Mean of the triplicate measures of Right Step Length (m)}

Additional analyses may be performed as appropriate.

4.7.2.2. Part 2

Primary Efficacy Analysis (Double-Blind Phase)

MRI: Total Muscle Volume

Individual total muscle volume data will be listed. Descriptive statistics will be provided by treatment group and scheduled time for raw data and changes from baseline (absolute and percent change). Absolute and percent changes from baseline will be provided for each side (left and right) as well as the average of the absolute change and average of the percent change for the left and right sides. This will be done for the double-blind, placebo-controlled component as well as for the open-label component.

For the double-blind component, the plot of mean (± SEM) for the average of left and right side percent change in total muscle volume from baseline by scheduled day and treatment group for each muscle group (BB and TA) during the double-blind period as well as during the open-label extension period will be provided.

For the double-blind component, an ANCOVA model will be fitted in order to assess the effect of ACE-083 on the Day 190 average percent change from baseline in total muscle volume for the left and right sides. Covariates to be included in the model are MUSCLE, TREATMENT, BASELINE, MRC-MMT GRADE CATEGORY, MUSCLE*TREATMENT, and BASELINE*MUSCLE where:

- MUSCLE represents the muscle treated (BB or TA)
- TREATMENT represents the treatment group (ACE-083 or placebo)
- BASELINE represents the baseline total muscle volume (average of left and right sides)
- MRC-MMT GRADE CATEGORY represents the randomization stratification factor (3 to 4- or 4- to 4+)
- MUSCLE*TREATMENT represents the muscle-by-treatment interaction
- BASELINE*MUSCLE represents the baseline-by-muscle interaction

The effect of ACE-083 on the percent change in TMV from baseline of the injected TA or BB at Day 190 versus placebo will be estimated using the ANCOVA model described above and tested using a 0.1 significance level (2-sided) separately for TA and BB. The least squares mean estimate along with the corresponding 90% confidence interval will be provided separately for TA and BB. In addition, the 95% confidence interval will also be provided as well as a bar chart

of the least squares mean estimates (± SEM) for each of the treatment groups separately for TA and BB.

A mixed model repeated measures (MMRM) analysis will be performed to assess the sensitivity to the ANCOVA analysis described above. An unstructured covariance structure will be used to model the within-patient covariance across visits. Other covariance structures will be considered if the unstructured covariance fails. The covariates to be included consist of all of the covariates described above for the ANCOVA with the addition of the following additional covariates: VISIT where VISIT represents the scheduled visit day post first dose of MRI assessment (D43, D106, D190), MUSCLE*VISIT, TREATMENT*VISIT, and MUSCLE*TREATMENT*VISIT.

For both the ANCOVA and MMRM analyses, multiple imputations will be performed for missing data. Twenty imputations will be performed. The seed to be used is specified in Section 5. The assumption of the missing data mechanism will be missing at random (MAR) unless the data suggest otherwise. The missing data pattern (monotone or arbitrary) will also be evaluated and the regression method for imputing missing data will be utilized for each scheduled timepoint with missing data. Covariates to be included when imputing missing data include: MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, MUSCLE*TREATMENT, and TMV percent change data at scheduled timepoints prior to the timepoint at which TMV percent change is being imputed.

The table below illustrates the covariates that should be used for imputing missing data:

Data to be Imputed	**Covariates to use in Multiple Imputation Regression Model**
Baseline TMV	MRC-MMT GRADE CATEGORY, MUSCLE, TREATMENT, MUSCLE*TREATMENT
Day 43 Percent change in TMV from baseline	MRC-MMT GRADE CATEGORY, MUSCLE, TREATMENT, MUSCLE*TREATMENT, BASELINE
Day 106 Percent change in TMV from baseline	MRC-MMT GRADE CATEGORY, MUSCLE, TREATMENT, MUSCLE*TREATMENT, BASELINE, Day 43 TMV percent change
Day 190 Percent change in TMV from baseline	MRC-MMT GRADE CATEGORY, MUSCLE, TREATMENT, MUSCLE*TREATMENT, BASELINE, Day 43 TMV percent change, Day 106 TMV percent change

Analysis findings will be presented for Day 190 percent change data where missing data is not imputed (observed data) as well as for Day 190 percent change data where multiple imputations are performed for missing data to assess the impact of the missing data on the observed study data. The analyses where multiple imputations are performed are considered to be primary.

For BB patients, the above analyses will be repeated using the patient's "preferred" side used for the PUL test for patients.

The Per Protocol Set will be used for the primary analysis. The analyses described above will also be repeated for the Full Analysis Set.

Additional analyses may be performed as appropriate.

MRI: Calculated Contractile Muscle Volume

Individual calculated contractile muscle volume data will be listed. Descriptive statistics will be provided by treatment group and scheduled time for raw data and changes from baseline (absolute and percent change). Absolute and percent changes from baseline will be provided for each side (left and right) as well as the average of the left and right sides. This will be done for the double-blind, placebo-controlled component as well as for the open-label component.

For the double-blind component, the plot of mean (± SEM) for the average of left and right side percent change in calculated contractile muscle volume from baseline (average of left and right sides) by scheduled day and treatment group for each muscle group (BB and TA) during the double-blind period as well as during the open-label extension period.

For the double-blind component, an ANCOVA model will be fitted in order to assess the effect of ACE-083 on the Day 190 average percent change from baseline in calculated contractile muscle volume for the left and right sides. Covariates to be included in the model are: MUSCLE, TREATMENT, BASELINE, MRC-MMT GRADE CATEGORY, MUSCLE*TREATMENT, and BASELINE*MUSCLE where:

- MUSCLE represents the muscle treated (BB or TA)
- TREATMENT represents the treatment group (ACE-083 or placebo)
- BASELINE represents the baseline calculated contractile muscle volume (average of left and right sides)
- MRC-MMT GRADE CATEGORY represents the randomization stratification factor (3 to 4- or 4- to 4+)
- MUSCLE*TREATMENT represents the muscle-by-treatment interaction
- BASELINE*MUSCLE represents the baseline-by-muscle interaction

The effect of ACE-083 on the Day 190 average percent change in calculated contractile muscle volume from baseline of the injected TA or BB versus placebo will be estimated using the ANCOVA model described above and tested using a 0.1 significance level (2-sided) separately for TA and BB. The least square mean estimate along with the corresponding 90% confidence interval will be provided separately for TA and BB. In addition, the 95% confidence interval will also be provided as well as a bar chart of the least squares mean estimates (± SEM) for each of the treatment groups separately for TA and BB.

A mixed model repeated measures (MMRM) analysis will be performed to assess the sensitivity to the ANCOVA analysis described above. An unstructured covariance structure will be used to model the within-patient covariance across visits. Other covariance structures will be considered if the unstructured covariance fails. The covariates to be included consist of all of the covariates described above for the ANCOVA with the addition of the following additional covariates: VISIT where VISIT represents the scheduled visit day post first dose of MRI assessment (D43, D106, D190), MUSCLE*VISIT, TREATMENT*VISIT, and MUSCLE*TREATMENT*VISIT.

For both the ANCOVA and MMRM analyses, multiple imputations will be performed for missing data. Twenty imputations will be performed. Imputations will be performed separately for BB and TA patients. The seed to be used is specified in Section 6. The assumption of the missing data mechanism will be missing at random (MAR) unless the data suggest otherwise. The missing data pattern (monotone or arbitrary) will also be evaluated and the regression method for imputing missing data will be utilized for each scheduled timepoint with missing data. Covariates to be included when imputing missing data include: MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, and CCMV percent change data at scheduled timepoints prior to the timepoint at which CCMV percent change is being imputed.

The table below illustrates the covariates that should be used for imputing missing data:

Data to be Imputed	**Covariates to use in Multiple Imputation Regression Model**
Baseline CCMV	MRC-MMT GRADE CATEGORY, TREATMENT, MUSCLE*TREATMENT
Day 43 Percent change in CCMV from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE
Day 106 Percent change in CCMV from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, Day 43 CCMV percent change
Day 190 Percent change in CCMV from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, Day 43 CCMV percent change, Day 106 CCMV percent change

Analysis findings will be presented for Day 190 percent change data where missing data is not imputed (observed data) as well as for Day 190 percent change data where multiple imputations are performed for missing data to assess the impact of the missing data on the observed study data. The analyses where multiple imputations are performed are considered to be primary.

For BB patients, the above analyses will be repeated using the patient's "preferred" side used for the PUL test for patients.

The Per Protocol Set will be used for the primary analysis. The analyses described above will also be repeated for the Full Analysis Set.

Additional analyses may be performed as appropriate.

MRI: Fat Fraction

For fat fraction data, the absolute change from baseline (by side and average of left and right sides) of the injected TA or BB will be summarized for each scheduled time. Such summaries will also be done for raw data and percent change from baseline.

For the double-blind component, an ANCOVA model will be fitted in order to assess the effect of ACE-083 on the Day 190 absolute change in fat fraction from baseline data (average of left and right sides). Covariates to be included in the model are MUSCLE, TREATMENT, MRC-MMT GRADE CATEGORY, BASELINE, BASELINE*MUSCLE and MUSCLE*TREATMENT where BASELINE represents the baseline fat fraction (average of left

and right sides) and each of the other covariates are defined similarly as what was done for the primary efficacy analysis.

The effect of ACE-083 on the Day 190 absolute change of fat fraction from baseline (average of left and right sides) of the injected TA or BB versus placebo will be estimated using the ANCOVA model described above and tested using a 0.1 significance level (2-sided) separately for TA and BB. The least-square mean estimate along with the corresponding 90% confidence interval will be provided. In addition, the 95% confidence interval will also be provided separately for TA and BB.

A mixed model repeated measures (MMRM) analysis will be performed to assess the sensitivity to the ANCOVA analysis described above. An unstructured covariance structure will be used to model the within-patient covariance across visits. Other covariance structures will be considered if the unstructured covariance fails. The covariates to be included consist of all of the covariates described above for the ANCOVA with the addition of the following additional covariates: VISIT where VISIT represents the scheduled visit day post first dose of MRI assessment (D43, D106, D190), MUSCLE*VISIT, TREATMENT*VISIT, and MUSCLE*TREATMENT*VISIT.

For both the ANCOVA and MMRM analyses, multiple imputations will be performed for missing data. Twenty imputations will be performed. The seed to be used is specified in Section 6. The assumption of the missing data mechanism will be missing at random (MAR) unless the data suggest otherwise. The missing data pattern (monotone or arbitrary) will also be evaluated and the regression method for imputing missing data will be utilized for each scheduled timepoint with missing data. Covariates to be included when imputing missing data include: MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, and FF absolute change data at scheduled timepoints prior to the timepoint at which FF absolute change is being imputed.

The table below illustrates the covariates that should be used for imputing missing data:

Data to be Imputed	**Covariates to use in Multiple Imputation Regression Model**
Baseline FF	MRC-MMT GRADE CATEGORY, TREATMENT,
Day 43 Absolute change in FF from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE
Day 106 Absolute change in FF from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, Day 43 FF absolute change
Day 190 Absolute change in FF from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, Day 43 FF absolute change, Day 106 FF absolute change

Analysis findings will be presented for Day 190 percent change data where missing data is not imputed (observed data) as well as for Day 190 percent change data where multiple imputations are performed for missing data to assess the impact of the missing data on the observed study data. The analyses where multiple imputations are performed are considered to be primary.

For BB patients, the above analyses will be repeated using the patient's "preferred" side used for the PUL test for patients.

The Per Protocol Set will be used for the primary analysis. The analyses described above will also be repeated for the Full Analysis Set.

Additional analyses may be performed as appropriate.

MRI: Calculated Intramuscular Fat

For calculated intramuscular fat data (volume and mass), the average percent change from baseline (left and right sides) of the injected TA or BB will be summarized for each scheduled time. Such summaries will also be done for raw data and change from baseline.

For the double-blind component, an ANCOVA model will be fitted in order to assess the effect of ACE-083 on the Day 190 average percent change in intramuscular fat from baseline (left and right sides). Covariates to be included in the model are MUSCLE, TREATMENT, MRC-MMT GRADE CATEGORY, BASELINE, BASELINE*MUSCLE, and MUSCLE*TREATMENT where BASELINE represents the baseline calculated intramuscular fat value and each of the other covariates are defined similarly as what was done for the primary analysis.

The effect of ACE-083 on the Day 190 average percent change of calculated intramuscular fat from baseline (left and right sides) of the injected TA or BB versus placebo will be estimated using the ANCOVA model described above and tested using a 0.1 significance level (2-sided) separately for TA and BB. The least-square mean estimate along with the corresponding 90% confidence interval will be provided. In addition, the 95% confidence interval will also be provided separately for TA and BB.

A mixed model repeated measures (MMRM) analysis will be performed to assess the sensitivity to the ANCOVA analysis described above. An unstructured covariance structure will be used to model the within-patient covariance across visits. Other covariance structures will be considered if the unstructured covariance fails. The covariates to be included consist of all of the covariates described above for the ANCOVA with the addition of the following additional covariates: VISIT where VISIT represents the scheduled visit day post first dose of MRI assessment (D43, D106, D190), MUSCLE*VISIT, TREATMENT*VISIT, and MUSCLE*TREATMENT*VISIT.

For both the ANCOVA and MMRM analyses, multiple imputations will be performed for missing data. Imputations will be performed separately for the BB and TA patients. Twenty imputations will be performed. The seed to be used is specified in Section 6. The assumption of the missing data mechanism will be missing at random (MAR) unless the data suggest otherwise. The missing data pattern (monotone or arbitrary) will also be evaluated and the regression method for imputing missing data will be utilized for each scheduled timepoint with missing data. Covariates to be included when imputing missing data include: MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, and CIMF (volume or mass) percent change data at scheduled timepoints prior to the timepoint at which CIMF (volume or mass) percent change is being imputed.

The table below illustrates the covariates that should be used for imputing missing data:

Data to be Imputed	Covariates to use in Multiple Imputation Regression Model
Baseline CIMF	MRC-MMT GRADE CATEGORY, TREATMENT
Day 43 Percent change in CIMF from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE
Day 106 Percent change in CIMF from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, Day 43 CIMF percent change
Day 190 Percent change in CIMF from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, Day 43 CIMF percent change, Day 106 CIMF percent change

Note: The above is to be applied for volume (CIMFV) as well as mass (CIMFM).

Analysis findings will be presented for Day 190 percent change data where missing data is not imputed (observed data) as well as for Day 190 percent change data where multiple imputations are performed for missing data to assess the impact of the missing data on the observed study data. The analyses where multiple imputations are performed are considered to be primary.

The Per Protocol Set will be used for the primary analysis. The analyses described above will also be repeated for the Full Analysis Set.

Additional analyses may be performed as appropriate.

Muscle Strength (MVIC)

Descriptive statistics will be provided by treatment group and scheduled time for raw data and changes from baseline (absolute and percent change). Absolute and percent changes from baseline will be provided for each side (left and right) as well as the average of the absolute change and average of the percent change for the left and right sides. This will be done for the double-blind placebo-controlled component as well as for the open-label component.

A plot of the mean (± SEM) of the average percent change from baseline (left and right sides) by scheduled day and treatment group will also be provided.

An ANCOVA model will be fitted in order to assess the effect of ACE-083 on the average Day 190 percent change in elbow flexion MVIC (left and right sides for patients receiving study drug in the biceps brachii muscle) from baseline data (average of left and right sides). Covariates to be included in the model are TREATMENT, BASELINE, and MRC-MMT GRADE CATEGORY, where BASELINE refers to the baseline MVIC (average of left and right sides) and each of the other covariates are defined similarly as what was done for the primary efficacy analysis.

A similar ANCOVA model will be fitted in order to assess the effect of ACE-083 on the Day 190 average percent change in ankle dorsiflexion MVIC (left and right sides for patients receiving study drug in the tibialis anterior muscle) from baseline data (average of left and right sides). Covariates will be the same set as described above for the elbow flexion MVIC analysis.

The effect of ACE-083 on the Day 190 average percent change in the MVIC from baseline of the injected TA or BB versus placebo will be estimated using the ANCOVA models described above

and tested using a 0.1 significance level (2-sided) separately for TA and BB. For TA and BB groups, the least squares mean estimate along with the corresponding 90% confidence interval will be provided. In addition, the 95% confidence interval will also be provided.

A mixed model repeated measures (MMRM) analysis will be performed to assess the sensitivity to the ANCOVA analysis described above. An unstructured covariance structure will be used to model the within-patient covariance across visits. Other covariance structures will be considered if the unstructured covariance fails. The covariates to be included consist of all of the covariates described above for the ANCOVA with the addition of the following additional covariates: VISIT where VISIT represents the scheduled visit day post first dose of MRI assessment and TREATMENT*VISIT.

For both the ANCOVA and MMRM analyses, multiple imputations will be performed for missing data. Twenty imputations will be performed. The seed to be used is specified in Section 6 . The assumption of the missing data mechanism will be missing at random (MAR) unless the data suggest otherwise. The missing data pattern (monotone or arbitrary) will also be evaluated and the regression method for imputing missing data will be utilized for each scheduled timepoint with missing data. Covariates to be included when imputing missing data include: MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, and MVIC percent change data at scheduled timepoints prior to the timepoint at which MVIC percent change is being imputed.

The table below illustrates the covariates that should be used for imputing missing data for selected timepoints:

Data to be Imputed	**Covariates to use in Multiple Imputation Regression Model**
Baseline MVIC	MRC-MMT GRADE CATEGORY, TREATMENT
Day 22 Percent change in MVIC from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE
Day 43 Percent change in MVIC from baseline	MRC-MMT GRADE CATEGORY, MUSCLE, TREATMENT, MUSCLE*TREATMENT, BASELINE, Day 22 MVIC percent change
Day 190 Percent change in MVIC from baseline	MRC-MMT GRADE CATEGORY, MUSCLE, TREATMENT, MUSCLE*TREATMENT, BASELINE, Days 22, 43, 64, 85, 106, and 148 MVIC percent change from baseline

Note: The above is to be applied for elbow flexion MVIC (BB treated patients) and ankle dorsiflexion (TA treated patients).

Analysis findings will be presented for Day 190 percent change data where missing data is not imputed (observed data) as well as for Day 190 percent change data where multiple imputations are performed for missing data to assess the impact of the missing data on the observed study data. The analyses where multiple imputations are performed are considered to be primary.

For BB patients, the above analyses will be repeated using the patient's "preferred" side used for the PUL test for patients.

The Per Protocol Set will be used for the primary analysis. The analyses described above will also be repeated for the Full Analysis Set.

MRC-MMT Decimal Score

Descriptive statistics will be provided for raw and average percent change from baseline (left and right sides) for the MRC-MMT decimal score where MRC-MMT decimal score represents the conversion of the MRC-MMT grade to the decimal version as follows: 5 = 5.0, 5- = 4.67, 4+ = 4.33, 4 = 4.0, 4- = 3.67, 3+ = 3.33, 3 = 3.0, 3- = 2.67, 2+ = 2.33, 2 = 2.0, 2- = 1.67, 1+ = 1.33, 1 = 1.0. The average percent change from baseline (left and right sides) is derived by computing the percent change from baseline separately for the left and right sides and then taking the average.

An ANCOVA model will be fitted in order to assess the effect of ACE-083 on the Day 190 average percent change in MRC-MMT score (for ankle dorsiflexion {TA patients} or for elbow flexion {BB patients}) versus placebo will be estimated using the following covariates: TREATMENT and BASELINE MRC-MMT DECIMAL SCORE, where TREATMENT is defined similarly as what was done for the primary analysis.

No imputations for missing data will be performed.

In addition, a summary table will be provided that will summarize the number and percentage of patients by treatment group for each side (left and right side) as well as the weaker of the two sides that

- Improved at least one grade at Day 190 compared to the baseline grade
- No change in MRC-MMT grade at Day 190 compared to the baseline grade
- Decreased at least one grade at Day 190 compared to the baseline grade

An improvement of at least one grade means that the patient moved from one grade to any higher grade on the range of possible values for the MRC-MMT grade (e.g. from a 4- to a 4).

A decrease of at least one grade means that the patient moved from one grade to any lower grade on the range of possible values for the MRC-MMT grade (e.g. from a 4 to a 4-).

Additional analyses may be performed as appropriate.

Motor Function (Lower Leg): 10-Meter Walk/Run Test (TA Muscle Group)

Individual data will be listed.

Descriptive statistics of raw data as well as absolute and percent change from baseline will be provided by cohort (for Part 1) and treatment group (for Part 2) for each scheduled time. A plot of mean ± SEM will also be provided for the percent change from baseline for each treatment group.

An ANCOVA model will be fitted in order to assess the effect of ACE-083 on the Day 190 percent change in the time to complete the 10-meter walk/run test from baseline data. Covariates to be included in the model are TREATMENT, HEIGHT, MRC-MMT GRADE CATEGORY, and BASELINE where BASELINE represents the baseline time to complete the 10 meter

walk/run test, HEIGHT is the height of the patient in centimeters, and each of the other covariates are defined similarly as what was done for the primary efficacy analysis.

The effect of ACE-083 on the Day 190 percent change in the time to complete the 10-meter walk/run test from versus placebo will be estimated using the ANCOVA model described above and tested using a 0.1 significance level (2-sided). The least-squares mean estimate along with the corresponding 90% confidence interval will be provided. In addition, the 95% confidence interval will also be provided as well as a bar chart of the least squares mean estimates (± SEM) for each of the treatment groups.

An ANCOVA model will also be fitted in order to assess the effect of ACE-083 on the Day 190 absolute change in the time to complete the 10-meter walk/run test from baseline data. Covariates to be included in the model are TREATMENT, HEIGHT, MRC-MMT GRADE CATEGORY, and BASELINE where BASELINE represents the baseline time to complete the 10 meter walk/run test, HEIGHT is the height of the patient in centimeters, and each of the other covariates are defined similarly as what was done for the primary efficacy analysis.

The effect of ACE-083 on the Day 190 absolute change in the time to complete the 10-meter walk/run test from versus placebo will be estimated using the ANCOVA model described above and tested using a 0.1 significance level (2-sided). The least-squares mean estimate along with the corresponding 90% confidence interval will be provided. In addition, the 95% confidence interval will also be provided as well as a bar chart of the least squares mean estimates (± SEM) for each of the treatment groups.

A mixed model repeated measures (MMRM) analysis will be performed to assess the sensitivity to the ANCOVA analysis described above. An unstructured covariance structure will be used to model the within-patient covariance across visits. Other covariance structures will be considered if the unstructured covariance fails. The covariates to be included consist of all of the covariates described above for the ANCOVA with the addition of the following additional covariates: VISIT where VISIT represents the scheduled visit day post first dose of MRI assessment and TREATMENT*VISIT.

For both the ANCOVA and MMRM analyses, multiple imputations will be performed for missing data. Twenty imputations will be performed. The seed to be used is specified in Section 6. The assumption of the missing data mechanism will be missing at random (MAR) unless the data suggest otherwise. The missing data pattern (monotone or arbitrary) will also be evaluated and the regression method for imputing missing data will be utilized for each scheduled timepoint with missing data. Covariates to be included when imputing missing data include: MRC-MMT GRADE CATEGORY, TREATMENT, HEIGHT, BASELINE, and 10mW/R percent change data at scheduled timepoints prior to the timepoint at which 10mW/R percent change is being imputed.

The table below illustrates the covariates that should be used for imputing missing data for selected timepoints:

Data to be imputed	Covariates to use in multiple imputation regression model
Baseline time to complete 10mW/R	MRC-MMT GRADE CATEGORY, TREATMENT, HEIGHT
Day 22 percent change in time to complete 10mW/R from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, HEIGHT, BASELINE
Day 43 percent change in time to complete 10mW/R from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, HEIGHT, BASELINE, Day 22 10mW/R percent change
Day 190 Percent change in time to complete 10mW/R from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, HEIGHT, BASELINE, Days 22, 43, 64, 85, 106, 148, and 169 10mW/R percent change from baseline

Analysis findings will be presented for Day 190 percent change data where missing data is not imputed (observed data) as well as for Day 190 percent change data where multiple imputations are performed for missing data to assess the impact of the missing data on the observed study data. The analyses where multiple imputations are performed are considered to be primary.

The above multiple imputation will also be done for missing absolute changes from baseline similarly to what is outlined above for the missing percent changes from baseline.

The Per Protocol Set will be used for the primary analysis. The analyses described above will also be repeated for the Full Analysis Set.

Additional analyses may be performed as appropriate.

Motor Function (Lower Leg): 6-Minute Walk Test (TA Muscle Group)

Individual data will be listed.

Descriptive statistics of raw data as well as changes from baseline (absolute and percent change) will be provided by cohort (for Part 1) and by treatment group (for Part 2) by scheduled time separately for cumulative distance recorded at 1 minute, 2 minutes, 3 minutes, 4 minutes, 5 minutes, and 6 minutes. A plot of mean ± SEM for the cumulative distance recorded at 6 minutes will also be provided for the percent change from baseline for each treatment group.

An ANCOVA model will be fitted in order to assess the effect of ACE-083 on the Day 190 percent change in the cumulative distance recorded at 6 minutes from baseline data paying particular attention to the appropriate choice of covariance structure. Covariates to be included in the model are TREATMENT, HEIGHT, MRC-MMT GRADE CATEGORY, and BASELINE where BASELINE represents the baseline distance walked in 6 minutes, HEIGHT represents the patient's height in centimeters, and each of the other covariates are defined the same as what was done for the primary efficacy analysis.

The effect of ACE-083 on the Day 190 percent change in the cumulative distance recorded at 6 minutes from baseline versus placebo will be estimated using the ANCOVA model described

above and tested using a 0.1 significance level (2-sided). The least-squares mean estimate along with the corresponding 90% confidence interval will be provided. In addition, the 95% confidence interval will also be provided as well as a bar chart of the least squares mean estimates (± SEM) for each of the treatment groups.

An ANCOVA model will be also be fitted in order to assess the effect of ACE-083 on the Day 190 absolute change in the cumulative distance recorded at 6 minutes from baseline data. Covariates to be included in the model are TREATMENT, HEIGHT, MRC-MMT GRADE CATEGORY, and BASELINE where BASELINE represents the baseline distance walked in 6 minutes, HEIGHT represents the height of the patient in centimeters, and each of the other covariates are defined the same as what was done for the primary efficacy analysis.

The effect of ACE-083 on the Day 190 absolute change in the cumulative distance recorded at 6 minutes from baseline versus placebo will be estimated using the ANCOVA model described above and tested using a 0.1 significance level (2-sided). The least-squares mean estimate along with the corresponding 90% confidence interval will be provided. In addition, the 95% confidence interval will also be provided as well as a bar chart of the least squares mean estimates (± SEM) for each of the treatment groups.

A mixed model repeated measures (MMRM) analysis will be performed to assess the sensitivity to the ANCOVA analysis described above. An unstructured covariance structure will be used to model the within-patient covariance across visits. Other covariance structures will be considered if the unstructured covariance fails. The covariates to be included consist of all of the covariates described above for the ANCOVA with the addition of the following additional covariates: VISIT where VISIT represents the scheduled visit day post first dose of MRI assessment and TREATMENT*VISIT.

For both the ANCOVA and MMRM analyses, multiple imputations will be performed for missing data. Twenty imputations will be performed. The seed to be used is specified in Section 6. The assumption of the missing data mechanism will be missing at random (MAR) unless the data suggest otherwise. The missing data pattern (monotone or arbitrary) will also be evaluated and the regression method for imputing missing data will be utilized for each scheduled timepoint with missing data. Covariates to be included when imputing missing data include: MRC-MMT GRADE CATEGORY, TREATMENT, HEIGHT, BASELINE, and 6MWD percent change data at scheduled timepoints prior to the timepoint at which 10mW/R percent change is being imputed.

The table below illustrates the covariates that should be used for imputing missing data for selected timepoints:

Data to be imputed	Covariates to use in multiple imputation regression model
Baseline 6MWD	MRC-MMT GRADE CATEGORY, TREATMENT, HEIGHT
Day 22 percent change in 6MWD from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, HEIGHT, BASELINE
Day 43 percent change in 6MWD from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, HEIGHT, BASELINE, Day 22 6MWD percent change
Day 190 Percent change in 6MWD from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, HEIGHT, BASELINE, Days 22, 43, 64, 85, 106, 148, and 169 6MWD percent change from baseline

Analysis findings will be presented for Day 190 percent change data where missing data is not imputed (observed data) as well as for Day 190 percent change data where multiple imputations are performed for missing data to assess the impact of the missing data on the observed study data.

The above multiple imputation will also be done for missing absolute changes from baseline similarly to what is outlined above for the missing percent changes from baseline. The analyses where multiple imputations are performed are considered to be primary.

The Per Protocol Set will be used for the primary analysis. The analyses described above will also be repeated for the Full Analysis Set.

In addition, the ANCOVA analyses described above for the 6MWD will be repeated for the cumulative distance walked at 2 minutes. This analysis is considered exploratory and it is not planned to impute missing data. Additional analyses may be performed as appropriate.

Motor Function (Lower Leg): 4-Stair Climb (TA Muscle Group)

Individual data will be listed.

Descriptive statistics of raw data as well as absolute and percent changes from baseline will be provided by cohort (for Part 1) and by treatment group (for Part 2) for time to ascend as well as time to descend data.

An ANCOVA model will be fitted in order to assess the effect of ACE-083 on the Day 190 percent change in the time to ascend 4-stairs from baseline data. Covariates to be included in the model are TREATMENT, MRC-MMT GRADE CATEGORY, and BASELINE where BASELINE represents the baseline time to ascend 4-stairs, and each of the other covariates are defined the same as what was done for the primary efficacy analysis.

The effect of ACE-083 on the Day 190 percent change in the time to ascend 4-stairs from baseline versus placebo will be estimated using the ANCOVA model described above and tested using a 0.1 significance level (2-sided). The least-squares mean estimate along with the corresponding 90% confidence interval will be provided. In addition, the 95% confidence

interval will also be provided as well as a bar chart of the least squares mean estimates (± SEM) for each of the treatment groups.

An ANCOVA model will be fitted in order to assess the effect of ACE-083 on the Day 190 percent change in the time to descend 4-stairs from baseline data. An unstructured covariance structure will be used to model the within-patient covariance across visits. Other covariance structures will be considered if the unstructured covariance fails. Covariates to be included in the model are TREATMENT, MRC-MMT GRADE CATEGORY, and BASELINE where BASELINE represents the baseline time to descend 4-stairs, and each of the other covariates are defined the same as what was done for the primary efficacy analysis.

The effect of ACE-083 on the Day 190 percent change in the time to descend 4-stairs from baseline versus placebo will be estimated using the ANCOVA model described above and tested using a 0.1 significance level (2-sided). The least-squares mean estimate along with the corresponding 90% confidence interval will be provided. In addition, the 95% confidence interval will also be provided as well as a bar chart of the least squares mean estimates (± SEM) for each of the treatment groups.

A mixed model repeated measures (MMRM) analysis will be performed to assess the sensitivity to the ANCOVA analyses described above. The covariates to be included consist of all of the covariates described above for the ANCOVA with the addition of the following additional covariates: VISIT where VISIT represents the scheduled visit day post first dose of MRI assessment and TREATMENT*VISIT.

For both the ANCOVA and MMRM analyses, multiple imputations will be performed for missing data. Twenty imputations will be performed. The seed to be used is specified in Section 6 . The assumption of the missing data mechanism will be missing at random (MAR) unless the data suggest otherwise. The missing data pattern (monotone or arbitrary) will also be evaluated and the regression method for imputing missing data will be utilized for each scheduled timepoint with missing data. Covariates to be included when imputing missing data include: MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, and 4-stair ascend (or descend) time percent change data at scheduled timepoints prior to the timepoint at which 4-stair ascend (or descend) time percent change is being imputed.

The table below illustrates the covariates that should be used for imputing missing data for selected timepoints:

Data to be Imputed	Covariates to use in Multiple Imputation Regression Model
Baseline time to ascend (or descend) 4 stairs	MRC-MMT GRADE CATEGORY, TREATMENT
Day 22 percent change in time to ascend (or descend) 4 stairs from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE
Day 43 percent change in time to ascend (or descend) 4 stairs from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, Day 22 4-stair climb ascend (or descend) time percent change
Day 190 Percent change in time to ascend (or descend) 4 stairs from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, Days 22, 43, 64, 85, 106, 148, and 169 4-stair climb ascend (or descend) time percent change from baseline

Analysis findings will be presented for Day 190 percent change data where missing data is not imputed (observed data) as well as for Day 190 percent change data where multiple imputations are performed for missing data to assess the impact of the missing data on the observed study data. The analyses where multiple imputations are performed are considered to be primary.

The Per Protocol Set will be used for the primary analysis. The analyses described above will also be repeated for the Full Analysis Set.

Additional analyses may be performed as appropriate.

Motor Function (Upper Arm): Performance of Upper Limb (PUL) [Middle Level Elbow Dimension] – (BB Muscle Group)

Individual data will be listed.

Descriptive statistics will be provided by treatment group for those receiving study drug in the biceps brachii muscle for the following components from the mid-level PUL:

- Composite (or average) of time to complete the four timed tasks from the mid-level PUL
- Time to lift 5 light cans
- Time to stack 5 light cans
- Time to lift 5 heavy cans
- Time to stack 5 heavy cans
- Mid-level elbow dimension total score

For the composite (or average) of times to complete the four timed tasks from the mid-level PUL, an ANCOVA model will be fitted in order to assess the effect of ACE-083 on the Day 190 percent change from baseline data. Covariates to be included in the model are TREATMENT, MRC-MMT GRADE CATEGORY, and BASELINE where BASELINE represents the baseline

PUL composite time and each of the other covariates are defined the same as what was done for the primary analysis.

The effect of ACE-083 on the Day 190 percent change in the mid-level PUL composite time from baseline versus placebo will be estimated using the ANCOVA model described above and tested using a 0.1 significance level (2-sided). The least-squares mean estimate along with the corresponding 90% confidence interval will be provided. In addition, the 95% confidence interval will also be provided as well as a bar chart of the least squares mean estimates (± SEM) for each of the treatment groups.

A mixed model repeated measures (MMRM) analysis will be performed to assess the sensitivity to the ANCOVA analysis described above. An unstructured covariance structure will be used to model the within-patient covariance across visits. Other covariance structures will be considered if the unstructured covariance fails. The covariates to be included consist of all of the covariates described above for the ANCOVA with the addition of the following additional covariates: VISIT where VISIT represents the scheduled visit day post first dose of MRI assessment and TREATMENT*VISIT.

For both the ANCOVA and MMRM analyses, multiple imputations will be performed for missing data. Twenty imputations will be performed. The seed to be used is specified in Section 6. The assumption of the missing data mechanism will be missing at random (MAR) unless the data suggest otherwise. The missing data pattern (monotone or arbitrary) will also be evaluated and the regression method for imputing missing data will be utilized for each scheduled timepoint with missing data. Covariates to be included when imputing missing data include: MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, and PUL percent change data at scheduled timepoints prior to the timepoint at which PUL percent change is being imputed.

The table below illustrates the covariates that should be used for imputing missing data for selected timepoints:

Data to be imputed	**Covariates to use in multiple imputation regression model**
Baseline PUL	MRC-MMT GRADE CATEGORY, TREATMENT
Day 22 percent change in PUL from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE
Day 43 percent change in PUL from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, Day 22 PUL percent change
Day 190 Percent change in PUL from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, Days 22, 43, 64, 85, 106, 148, and 169 PUL percent change from baseline

Note: PUL means either the composite (mean) time to perform the 4 timed activities or the PUL mid-level dimension score.

Analysis findings will be presented for Day 190 percent change data where missing data is not imputed (observed data) as well as for Day 190 percent change data where multiple imputations are performed for missing data to assess the impact of the missing data on the observed study data. The analyses where multiple imputations are performed are considered to be primary.

The analyses described above will also be performed for the mid-level PUL dimension score.

The Per Protocol Set will be used for the primary analysis. The analyses described above will also be repeated for the Full Analysis Set.

Additional analyses may be performed as appropriate.

Motor function (upper arm): Performance of upper limb (PUL) [Upper level dimension] – (BB muscle group)

Individual data will be listed.

The number and percentage of subjects of weight used to perform each of the four components of the PUL upper level dimension (shoulder domain) will be presented by treatment group and scheduled time. The four components are:

- Shoulder abduction to shoulder height (elbow to shoulder level)
- Shoulder abduction above shoulder height (elbow to eye level)
- Shoulder flexion to shoulder height (elbow to shoulder level)
- Shoulder flexion above shoulder height (elbow to eye level)

Descriptive statistics will be provided by scheduled time for the raw PUL shoulder domain score (average PUL shoulder domain score of left and right sides) and changes from baseline (absolute and percent change) by treatment group sequence in the open-label phase of Part 2.

Additional analyses may be performed as appropriate.

Patient Reported Outcomes: Facioscapulohumeral Disease Health Index (FSHD-HI) Survey

Individual FSHD-HI scored data (total score and each of the subscale scores) will be listed.

Descriptive statistics of the raw data as well as for the absolute and percent change from baseline will be provided for the FSHD-HI total score as well as for each of the subscale scores.

Least square mean estimates of the effect of ACE-083 and corresponding 90% and 95% confidence intervals will be produced for the Day 190 absolute change data described above using an ANCOVA with MUSCLE, BASELINE, TREATMENT MRC-MMT GRADE CATEGORY, and MUSCLE*TREATMENT as covariates where MUSCLE, BASELINE, TREATMENT, and MRC-MMT GRADE CATEGORY are defined similarly as described above for other analyses. In addition, a bar chart of the least squares mean estimates (± SEM) by muscle for each of the treatment groups will be provided for the following FSHD-HI parameters: FSHD-HI total score, FSHD-HI Arm/Shoulder function (BB only), FSHD-HI Mobility (TA only), and FSHD-HI Activity limitation.

A mixed model repeated measures (MMRM) analysis will be performed to assess the sensitivity to the ANCOVA analysis described above. An unstructured covariance structure will be used to

model the within-patient covariance across visits. Other covariance structures will be considered if the unstructured covariance fails. The covariates to be included consist of all of the covariates described above for the ANCOVA with the addition of the following additional covariates: VISIT where VISIT represents the scheduled visit day post first dose assessment, MUSCLE*VISIT, TREATMENT*VISIT, and MUSCLE*TREATMENT*VISIT.

For both the ANCOVA and MMRM analyses, multiple imputations will be performed for missing data. Twenty imputations will be performed. Imputations will be performed separately for the BB and TA patients. The seed to be used is specified in Section 6. The assumption of the missing data mechanism will be missing at random (MAR) unless the data suggest otherwise. The missing data pattern (monotone or arbitrary) will also be evaluated and the regression method for imputing missing data will be utilized for each scheduled timepoint with missing data. Covariates to be included when imputing missing data include: MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE and FSHD-HI absolute change data at scheduled timepoints prior to the timepoint at which FSHD-HI absolute change is being imputed.

The table below illustrates the covariates that should be used for imputing missing data:

Data to be imputed	**Covariates to use in multiple imputation regression model**
Baseline FSHD-HI	MRC-MMT GRADE CATEGORY, TREATMENT
Day 22 absolute change from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE
Day 43 absolute change from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, Day 22 FSHD-HI absolute change
Day 190 absolute change from baseline	MRC-MMT GRADE CATEGORY, TREATMENT, BASELINE, Days 22, 43, 64, 85, 106, and 148 FSHD-HI absolute change

Analysis findings will be presented for Day 190 percent change data where missing data is not imputed (observed data) as well as for Day 190 percent change data where multiple imputations are performed for missing data to assess the impact of the missing data on the observed study data. The analyses where multiple imputations are performed are considered to be primary.

The Per Protocol Set will be used for the primary analysis. The analyses described above will also be repeated for the Full Analysis Set.

Additional analyses may be performed as appropriate.

Gait

Individual data will be listed.

Descriptive statistics will be provided for each type of measurement recorded (e.g., “General measures”, “Initial measures”, “Peak measures”, and “Average measures”) by treatment group and scheduled time.

For gait assessments that are reported in triplicates at a given visit for any patient and are assessed to be of good quality, the mean of these will be used for purposes of generating a single individual value for the patient at the scheduled visit which will then be summarized.

In addition, "Stride Length" will be derived using the following formula:

Stride Length (m) = {Mean of the triplicate measures of Left Step Length (m)} + {Mean of the triplicate measures of Right Step Length (m)}

Additional analyses may be performed as appropriate.

No imputations will be performed for missing gait data.

4.7.2.3. Part 2 Open-Label Extension

Individual data will be listed. The listing of such data will be collated with the data collected in the double-blind, placebo-controlled phase.

Descriptive statistics will be provided for raw data and changes (percent and absolute change) from the start of the open-label extension (Day 190) will be provided by treatment sequence for each scheduled time. The treatment sequence refers to the treatment sequence from double-blind to open label (i.e. ACE-083 □ ACE-083 or Placebo □ ACE-083). For MRI and strength data, such summaries will be provided by scheduled time for each side treated as well as the average of the left and right sides.

Additional analyses may be performed as appropriate.

Open-Label Extension vs. Double-Blind Placebo-Controlled Phase

Selected MRI, and FSHD-HI Total Score and FSHD-HI Activity Limitation Subscale Score

For total muscle volume, calculated contractile muscle volume, fat fraction, FSHD-HI total score, and FSHD-HI activity limitation subscale score, descriptive statistics will be provided for the Placebo □ ACE-083 treatment sequence for the difference in percent (or absolute) change observed between Day 190 and pre-first treatment baseline (1st 6 months) and the percent (or absolute) change observed between Day 358 and the open-label treatment baseline (2nd 6 months).

In addition, the effect of ACE-083 administered during the open-label extension compared to the treatment administered in the double-blind placebo-controlled phase will be examined for those receiving ACE-083 during the double-blind phase and separately for those receiving placebo during the double-blind phase by comparing the percent (or absolute) change observed between Day 190 and pre-first treatment baseline (1st 6 months) and Day 358 versus the open-label treatment baseline (2nd 6 months) using an ANCOVA model for each individual data parameter.

Covariates to be included into the model are MUSCLE, SEQUENCE, MRC-MMT GRADE CATEGORY, BASELINE, and MUSCLE*SEQUENCE where:

- MUSCLE refers to the muscle treated (i.e. BB or TA)
- SEQUENCE refers to the treatment sequence from double-blind to open-label (ACE-083 □ ACE-083 or Placebo □ ACE-083)

- MRC-MMT GRADE CATEGORY refers to the randomization stratification factor from the double-blind phase
- BASELINE refers to baseline from the double-blind phase
- MUSCLE*SEQUENCE refers to the muscle-by-sequence interaction

Least squares estimates of the SEQUENCE*MUSCLE effect and the corresponding 90% confidence interval will be provided and will be tested using a 0.1 significance level (2-sided). In addition, the least squares estimates for each sequence along with the corresponding 90% confidence interval will be provided separately for each muscle and will be tested using a 0.1 significance level (2-sided).

Multiple imputations will be performed for missing data. Twenty imputations will be performed. The seed to be used is specified in Section 6. The assumption of the missing data mechanism will be missing at random (MAR) unless the data suggest otherwise. The missing data pattern (monotone or arbitrary) will also be evaluated and the regression method for imputing missing data will be utilized for each scheduled timepoint with missing data. Covariates to be included when imputing missing data include: MRC-MMT GRADE CATEGORY, MUSCLE, SEQUENCE, BASELINE, MUSCLE*SEQUENCE, and percent (or absolute change) of the analysis variable at scheduled timepoints prior to the timepoint at which the analysis variable percent (or absolute) change is being imputed.

Analysis findings will be presented where missing data is not imputed (observed data) as well as for the case where multiple imputations are performed for missing data to assess the impact of the missing data on the observed study data. The analyses where multiple imputations are performed are considered to be primary.

The Per Protocol Set will be used for the primary analysis. The analyses described above will also be repeated for the Full Analysis Set.

Additional analyses may be performed as appropriate.

Selected Motor Function, FSHD-HI Arm/Shoulder Function Subscale Score, MVIC, MRC-MMT Score, FSHD-HI Mobility and Ambulation Subscale Score

For mid-level PUL composite time (BB patients only), 6MWD (TA patients only), time to complete the 10mW/R (TA patients only), 4-stair climb ascend time (TA patients only), 4-stair descend time (TA patients only), FSHD-HI arm/shoulder function subscale score (BB patients only), FSHD-HI mobility and ambulation subscale score (TA patients only), MVIC (elbow flexion – BB patients), MVIC (ankle dorsiflexion – TA patients), MRC-MMT score (elbow flexion – BB patients), MRC-MMT score (ankle dorsiflexion – TA patients), descriptive statistics will be provided for the Placebo ACE-083 treatment sequence for the difference in percent (or absolute) change observed between Day 190 and pre-first treatment baseline (1st 6 months) and the percent (or absolute) change observed between Day 358 and the open-label treatment baseline (2nd 6 months).

In addition, the effect of ACE-083 administered during the open-label extension compared to the treatment administered in the double-blind placebo-controlled phase will be examined for those receiving ACE-083 during the double-blind phase and separately for those receiving placebo during the double-blind phase by comparing the percent (or absolute) change observed between

Day 190 and pre-first treatment baseline (1st 6 months) and Day 358 versus open-label treatment baseline (2nd 6 months) using an ANCOVA model for each individual data parameter.

Covariates to be included into the model are SEQUENCE, MRC-MMT GRADE CATEGORY, HEIGHT (only for 6MWD and 10mW/R) and BASELINE where:

- SEQUENCE refers to the treatment sequence from double-blind to open-label (ACE-083 □ ACE-083 or Placebo □ ACE-083)
- MRC-MMT GRADE CATEGORY refers to the randomization stratification factor from the double-blind phase
- BASELINE refers to baseline from the double-blind phase
- HEIGHT refers to the patient's height (cm)

Least squares estimates for each sequence and 90% confidence intervals will be provided and will be tested using a 0.1 significance level (2-sided).

Multiple imputations will be performed for missing data. Twenty imputations will be performed. The seed to be used is specified in Section 6. The assumption of the missing data mechanism will be missing at random (MAR) unless the data suggest otherwise. The missing data pattern (monotone or arbitrary) will also be evaluated and the regression method for imputing missing data will be utilized for each scheduled timepoint with missing data. Covariates to be included when imputing missing data include: MRC-MMT GRADE CATEGORY, SEQUENCE, BASELINE, HEIGHT (only for 6MWD and 10mW/R) and percent (or absolute change) of the analysis variable at scheduled timepoints prior to the timepoint at which the analysis variable percent (or absolute) change is being imputed.

Analysis findings will be presented where missing data is not imputed (observed data) as well as for the case where multiple imputations are performed for missing data to assess the impact of the missing data on the observed study data. The analyses where multiple imputations are performed are considered to be primary.

The Per Protocol Set will be used for the primary analysis. The analyses described above will also be repeated for the Full Analysis Set.

Additional analyses may be performed as appropriate.

4.7.2.4. Subgroup Analyses (Part 2 Only)

Subgroups consist of the following:

- D4Z4 fragment size (< 23 kb; ≥ 23 kb)
- Dose per Calculated Contractile Muscle Mass (mg/g)
  - BB: (<2 .9; ≥ 2.9); TA: (< 3.5; ≥ 3.5)
- Exercise Program: (Some; None)
- Baseline FSHD-HI Total Score:
  - BB: (< median; ≥ median); TA: (< median; ≥ median)

Note that the median will be the median of the average of the FSHD-HI total score for the assessments performed at Screening Days -28 and -7 and Day 1 and will be determined separately for BB and TA patients.

- Baseline Fat Fraction (%)
  - BB: (< median; ≥ median); TA: (< median; ≥ median)

Note that the median will be determined separately for BB and TA patients.

- Knee Extension MRC-MMT Grade {weaker of two sides – TA patients only}
  - (2- to 4-; 4 to 5)
- Elbow Flexion MRC-MMT Grade {weaker of two sides – BB patients only}
  - (3 to 4-; 4 to 4+)
- Ankle Dorsiflexion MRC-MMT Grade {weaker of two sides – TA patients only}
  - (3 to 4-; 4 to 4+)

Subgroup analyses will be performed for the following parameters:

- Day 190 average percent change in total muscle volume from baseline
- Day 190 average percent change in calculated contractile muscle volume from baseline
- Day 190 average absolute change in fat fraction from baseline
- Day 190 average percent change in fat fraction from baseline
- Day 190 average percent change in elbow flexion MVIC from baseline
  - To be done for all subgroups above with the exception of "Knee Extension MRC-MMT Grade" and "Ankle Dorsiflexion MRC-MMT Grade" subgroups
- Day 190 average percent change in ankle dorsiflexion MVIC from baseline
  - To be done for all subgroups above with the exception of "Elbow Flexion MRC-MMT Grade"
- Day 190 percent change in 6MWD from baseline
  - To be done for all subgroups above with the exception of "Elbow Flexion MRC-MMT Grade"
- Day 190 percent change in time to complete 10mW/R from baseline
  - To be done for all subgroups above with the exception of "Elbow Flexion MRC-MMT Grade"
- Day 190 percent change in PUL composite time from baseline
  - To be done for all subgroups above with the exception of "Knee Extension MRC-MMT Grade" and "Ankle Dorsiflexion MRC-MMT Grade" subgroups
- Day 190 absolute change in FSHD-HI total score from baseline

- Day 190 absolute change in FSHD-HI mobility and ambulation subscale score from baseline
    - To be done only for the "Knee Extension MRC-MMT Grade" and "Ankle Dorsiflexion MRC-MMT Grade" subgroups
- Day 190 absolute change in FSHD-HI activity limitation subscale score from baseline
    - To be done only for the "Knee Extension MRC-MMT Grade", "Elbow Flexion MRC-MMT Grade", and "Ankle Dorsiflexion MRC-MMT Grade" subgroups.

Subgroup analyses will be performed using similar ANCOVA models described in previous sections for each subgroup category provided that the sample size is at least 4 within each muscle. Because of the reduced sample size within each subgroup category, it is not planned to include MRC-MMT GRADE CATEGORY into such models. For each subgroup category, the treatment effect will be estimated and tested using a 0.1 (two-sided) significance level with the corresponding 90% confidence interval separately for TA and BB. Bar charts of the least squares mean estimates (± SEM) for each of the treatment groups will be provided for the individual muscle.

For purposes of this study, findings of subgroup analyses are considered to yield hypothesis generating statements for future study. It is not planned to control the overall type-1 error.

Additional analyses may be performed as appropriate.

Multiple imputations will be performed for missing data using the same approach outlined in Section 4.7.2.2 or the parameter of interest.

4.7.2.5. Other Analyses

The number and percentage of patients belonging to each of the following criteria will be summarized by treatment group:

- Day 190 percent change in TMV from baseline ≥ 10%
- Day 190 percent change in 6MWD from baseline ≥ 10%
- Day 190 absolute change in 6MWD from baseline ≥ 30 m
- Day 190 percent change in time to complete 10mW/R from baseline ≥ 10%
- Day 190 absolute change in time to complete 10mW/R from baseline ≥ 0.5 sec.
- Day 190 percent change in 4-stair ascend time from baseline ≥10%
- Day 190 percent change in PUL composite (mean) time to perform 4 timed activities from baseline ≥ 10%
- Day 190 absolute change in FSHD-HI total score from baseline ≤ -8
- Day 190 absolute change in FSHD-HI arm/shoulder sub-score from baseline ≤ -8 (BB patients only)
- Day 190 absolute change in FSHD-HI mobility sub-score from baseline ≤ -8 (TA patients only)
- Day 190 absolute change in FSHD-HI activity limitation sub-score from baseline ≤ -8

For each criterion listed above, Fisher's exact test will be used to compare the proportions of patients belonging to the individual criterion between the two treatment groups using a 0.1 significance level.

4.8. Pharmacodynamic Data

4.8.1. Variables

Pharmacodynamic data consists of the following selected laboratory data (e.g. CTX and hemoglobin).

4.8.2. Analyses

4.8.2.1. Part 1

Individual pharmacodynamic data will be listed.

For each pharmacodynamic variable, raw data and changes from baseline will be summarized by dose cohort and scheduled time. Such summaries may be accompanied by a plot of mean ± SEM. No imputations for missing data will be performed.

Additional analyses may be performed as appropriate.

4.8.2.2. Part 2

Double-Blind Period

Individual pharmacodynamic data will be listed.

For each pharmacodynamic variable, raw data and changes from baseline will be summarized by treatment group and scheduled time. Such summaries may be accompanied by a plot of mean ± SEM. No imputations for missing data will be performed.

Additional analyses may be performed as appropriate.

Open-Label Period

Individual pharmacodynamic data will be listed.

For each pharmacodynamic variable, raw data and changes from baseline will be summarized by treatment sequence and scheduled time. Pooled summaries across treatment sequences will also be provided. Such summaries may be accompanied by a plot of mean ± SEM.

No imputations for missing data will be performed.

Additional analyses may be performed as appropriate.

4.9. Safety Data

4.9.1. Adverse Events

Adverse events (AEs) will be coded using MedDRA Version 19.1.

All AEs will be listed and will include but not necessarily be limited to the following: verbatim term, MedDRA preferred term, treatment group, severity, relationship to study medication,

action taken with respect to study medication, and whether or not the AE is treatment-emergent although not necessarily in that order.

Treatment-emergent AEs (TEAEs) are defined those AEs that start or worsen in intensity on or after the first study drug administration up to the end of the follow-up period (Day 141 [Part 1] / Day 393 [Part 2]). AEs classified as TEAEs will be summarized. Planned summaries include the following (for each muscle group unless otherwise specified):

- Overall summary of TEAEs by treatment group
- Summary of TEAEs by MedDRA System Organ Class and Preferred Term for each treatment group – Number of Patients
- Summary of TEAEs by MedDRA System Organ Class and Preferred Term for each treatment group – Number of Events
- Summary of TEAEs by MedDRA Preferred Term for each treatment group – Number of Patients
- Summary of TEAEs by MedDRA Preferred Term for each treatment group– Number of Events
- Summary of CTCAE Grade 3 or Higher TEAEs by MedDRA System Organ Class and Preferred Term for each treatment group – Number of Patients
- Summary of CTCAE Grade 3 or Higher TEAEs by MedDRA System Organ Class and Preferred Term for each treatment group – Number of Events
- Summmary of CTCAE Grade 3 or Higher TEAEs by MedDRA Preferred Term for each treatment group – Number of Patients
- Summmary of CTCAE Grade 3 or Higher TEAEs by MedDRA Preferred Term for each treatment group – Number of Events
- Summary of TEAEs possibly or probably related to study drug by MedDRA System Organ Class and Preferred Term for each treatment group – Number of Patients
- Summary of TEAEs possibly or probably related to study drug by MedDRA System Organ Class and Preferred Term for each treatment group– Number of Events
- Summary of TEAEs possibly or probably related to study drug by MedDRA Preferred Term for each treatment group – Number of Patients
- Summary of TEAEs possibly or probably related to study drug by MedDRA Preferred Term for each treatment group– Number of Events
- Summary of TEAEs by MedDRA Preferred Term by CTCAE Grade and Relationship to Drug for each treatment group – Number of Patients
- Summary of TEAEs by MedDRA Preferred Term by CTCAE Grade and Relationship to Drug for each treatment group – Number of Events
- Summary of Serious Adverse Events – Number of Patients

4.9.2. Clinical Laboratory Evaluations

The following table lists the planned clinical laboratory assessments:

Type of Assessment	Details
Hematology	Hemoglobin, hematocrit, platelet count, red blood cell count, white blood cell (WBC) count, and WBC differential
Chemistry	AST, ALT, lactate dehydrogenase (LDH) and isoenzymes 1-5, gamma-glutamyl transpeptidase (GGT), blood urea nitrogen (BUN), creatinine, creatine kinase (CK), myoglobin, aldolase, sodium, potassium, glucose, albumin, total bilirubin
Urine	Urinalysis by dipstick analysis (pH, specific gravity, protein, myoglobin, glucose, ketones, blood, leukocyte esterase, and nitrite)

In addition to the tests listed below, a urine pregnancy test is performed on all females of childbearing potential according to scheduled times listed in the protocol.

Descriptive statistics of raw data and change from baseline data will be provided by treatment group for each scheduled time.

Plots of individual patient data will be provided by treatment group and scheduled time for continuous laboratory data. Plots of mean ± SEM will be provided for raw data and change from baseline data for the following selected laboratory data: hemoglobin, glucose, aldolase, blood urea nitrogen (BUN), creatinine kinase (CK), creatinine, and myoglobin.

Shift tables describing out-of-range shifts from baseline (in frequency counts) will be created for post-dose time points.

Additional analyses may be performed as appropriate.

Vital Signs

Vital sign parameters consist of weight, heart rate, systolic and diastolic blood pressure.

Descriptive statistics will be provided for raw data and absolute change from baseline by scheduled time for each treatment group. Post-dose recheck values will not be used for calculation of descriptive statistics.

With the exception of weight, plots of individual patient data will be provided by treatment group and scheduled time. Plots of mean ± SEM will be provided for raw data and change from baseline data.

Additional analyses may be performed as appropriate.

Electrocardiogram

Twelve-lead electrocardiogram (ECG) parameters consist of the following: (heart rate, PR, QRS, QT, and QTc (Bazett correction).

Descriptive statistics will be provided for raw data and absolute change from baseline by scheduled time for each treatment group. Post-dose recheck values will not be used for calculation of descriptive statistics.

Additional analyses may be performed as appropriate.

4.9.3. Anti-Drug Antibodies

Individual anti-drug antibody (ADA) data will be listed.

The frequency and percentage of ADA responses will be summarized by treatment group and scheduled time.

The frequency and percentage of all patients testing positive for ADA at any point during the study (i.e. ADA prevalence) will be summarized by treatment group. This will include summaries of the prevalence ADA confirmed as "Anti-ACE-083" or "Anti-FST315". In addition, for ACE-083 ADA, a summary of the prevalence of ACE-083 ADA and titer summary (median, minimum, and maximum value) will be provided by scheduled visit and antibody follow-up visit (as applicable).

The ACE-083 ADA incidence is defined as the sum of both "treatment-induced" and "treatment-boosted" ACE-083 ADA positive subjects as a proportion of patients with at least one sample taken post study drug administration that is appropriate for ADA testing. Treatment induced ACE-083 ADA is defined as ACE-083 ADA developing de novo following study drug administration at any time following the first study drug administration in a patient without pre-existing ACE-083 ADA. Treatment boosted ACE-083 ADA is defined as a pre-existing ACE-083 ADA that was boosted to a higher level following study drug administration. ACE-083 ADA incidence will be summarized by treatment group.

Additional analyses may be performed as appropriate.

4.9.4. Other Safety Data

Not applicable.

4.10. Pharmacokinetic Data

Pharmacokinetic data will be summarized under the direction of the Acceleron Clinical Pharmacologist and summaries will be outlined in a separate analysis plan.

5. MODIFICATIONS FROM THE STATISTICAL SECTION OF THE PROTOCOL

The following modifications from the statistical section of the protocol have been implemented in the statistical analysis plan:

- For statistical analyses where confidence intervals for the least-squares mean are provided, the 95% confidence interval will be provided in addition to the 90% confidence interval.
- The “Safety Population” is now called the “Safety Set”.
- For muscle strength, “Peak force value” is now called “MVIC”.
- For motor function data (lower leg and upper arm functional assessments), strength (QMT by handheld device), gait, and FSHD-HI data whose pre-treatment assessments are performed up to three times prior to the first dose, the baseline is defined as the average of the non-missing values.
- The “Per Protocol Set” is defined as all patients enrolled/randomized in the study who have received at least one dose of study drug (includes placebo) with no major protocol violations.
- The last observation carried forward approach is not being performed in the statistical analyses. Standard multiple imputation will be performed to assess the impact of missing data on the observed data.

6. PROGRAMMING SPECIFICATIONS

Seed to Use for Multiple Imputations of Missing Data

The seed to be used for multiple imputations of MRI data is 1111.

The seed to be used for multiple imputations of FSHD-HI data is 2222.

The seed to be used for multiple imputations of TA functional data is 3333.

The seed to be used for multiple imputations of PUL data is 4444.

The seed to be used for multiple imputations of elbow flexion MVIC data is 5555.

The seed to be used for multiple imputations of ankle dorsiflexion MVIC data is 6666.

Representation of Scheduled Times in Listings

Because data for this study comes from multiple sources, such information will be standardized in the listings. The standardization is outlined below:

Lab (MRL), FSHD-HI, eCRF (long)	eCRF (short)	MRI	Mapping for Tables and Listings	Plots
Screening Day -28	SCN		S1D-28	-28
Screening Day -7	SCN2		S2D-7	-7
Cycle 1 Day 1	C1D1	DAY 1	C1D1	1
Cycle 1 Day 2	C1D2		C1D2	2
Cycle 1 Day 8	C1D8		C1D8	8
Cycle 2 Day 22	C2D22		C2D22	22
Cycle 3 Day 43	C3D43	DAY 43	C3D43	43
Cycle 4 Day 64	C4D64		C4D64	64
Cycle 5 Day 85	C5D85		C5D85	85
Cycle 5 Day 86	C5D86		C5D86	86
Follow-Up Day 106/ET[a]	ET	DAY 106	F1D106_ET	106
Cycle 6 Day 106[b]	C6D106	DAY 106	C6D106	106
Cycle 7 Day 127[b]	C7D127		C7D127	127
Follow-Up Day 141[a]	FUP	DAY 141	F2D141	141
Cycle 8 Day 148[b]	C8D148		C8D148	148

Lab (MRL), FSHD-HI, eCRF (long)	eCRF (short)	MRI	Mapping for Tables and Listings	Plots
Cycle 9 Day 169[b]	C9D169		C9D169	169
Cycle 10 Day 190[b]	C10D190	DAY 190	C10D190	190
Cycle 11 Day 211[b]	C11D211		C11D211	211
Cycle 12 Day 232[b]	C12D232		C12D232	232
Cycle 13 Day 253[b]	C13D253		C13D253	253
Cycle 14 Day 274[b]	C14D274		C14D274	274
Cycle 15 Day 295[b]	C15D295	DAY 295	C15D295	295
Cycle 16 Day 316[b]	C16D316		C16D316	316
Cycle 17 Day 337[b]	C17D337		C17D337	337
Day 358/ET[b]	ET	DAY 358	F1D358_ET	358
Follow-Up Day 393[b]	FUP	DAY 393	F2D393	393

[a] Applies to Part 1; [b] Applies to Part 2

Partial Date of Birth Entries

In the event that a patient does not have a complete date of birth present in the clinical database, the date of birth will be interpolated as follows:

- If the date of birth contains the month and year of birth but no value of day (i.e. day is missing or has value "UNK"), the value of day will be imputed to the 1st for purposes of any calculations that involve the complete birthdate of the patient (i.e. age).
- If the date of birth only contains the year but no value of month and day (i.e. "UNK-UNK-yyyy"), the birthdate will be imputed to "01-Jan-yyyy" where "yyyy" refers to the year that is reported for that patient for purposes of any calculations that involve the complete birthdate of the patient (i.e. age).

Partial AE Start Dates

The following rules will be utilized for 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 for the patient with the partial AE start date, then the month and day of the first dose date will be imputed.

  - Otherwise, the month will be imputed to January.
- If the day is unknown, then:
  - If the month and year match the first dose date, then the day of the first dose date will be imputed.
  - Otherwise, the day will be imputed to '01'.

Classification of Aes as Teaes

In general, the start of reporting of adverse events for an individual patient starts after receiving the first dose of study drug. However:

- If both the AE start and stop date exist and are before the first dose date of study drug, the AE will be classified as a pre-treatment AE and not considered treatment emergent.
- If the AE start date is on or after the first dose date of study drug, the AE will be considered a TEAE.
- If the AE start date is before the first dose date of study drug and the AE stop date is after the first dose of study drug and the AE worsened in intensity, the AE will be considered a TEAE.
- If the AE start date is missing and the stop date is before the first dose of study drug, the AE will be not be considered a TEAE.
- If the AE start date is missing and the stop date is after the first dose of study drug, the AE will be considered a TEAE.
- If the AE start and stop dates are missing, the AE will be considered a TEAE.

Unilateral vs. Bilateral (Part 1)

In Part 1 of this study, patients may be treated unilaterally (meaning that the patient receives his/her dose either on the left TA or BB muscle or the right TA or BB muscle) or bilaterally (meaning that the patient receives his/her dose on both the right and left sides).

For patients receiving ACE-083 unilaterally, the side treated will vary from patient to patient within a cohort. To properly identify the side treated, the dataset(s) that identify the dose administrations for the individual patient will be used.

Patients that Discontinue Early

For patients that discontinue early, end-of-treatment and follow-up assessments will be completed and analyzed as such.

7. APPENDICES

APPENDIX 1. SCHEDULE OF EVENTS

Part 1

	Screening Period		Treatment Period								Follow-Up Period	
			Cycle 1			Cycle 2	Cycle 3	Cycle 4	Cycle 5			
	Day -28	Day -7 (±3d)[15]	Day 1[1]	Day 2	Day 8 (±1d)[15]	Day 22[1] (±1d)[15]	Day 43[1] (±3d)[15]	Day 64[1] (±3d)[15]	Day 85[1] (±3d)[15]	Day 86	Day 106/ET[2] (±3d)[15]	Day 141/EOS (±3d)[15]
Informed consent	X											
Inclusion/exclusion criteria	X	X	X									
Urine pregnancy test[3]			X			X	X	X	X			
Medical history	X	X	X									
MMT assessment (MRC)	X											
Genetic testing[16]	X											
Physical examination[4]	X		X			X	X	X	X		X	
Injection site examination[5]			X	X	X	X	X	X	X	X	X	
Vital signs[6]	X	X	X		X	X	X	X			X	
Hematology[7]	X		X			X	X	X	X		X	
Chemistry[7]	X		X			X	X	X	X		X	
Urinalysis[7]	X		X				X	X			X	
PD Biomarkers	X		X			X	X	X			X	X
Anti-drug antibody			X		X	X	X	X	X		X	X[8]
Serum PK[9]			0, 2, 4, 6 h	X	X	X	X	X	0, 2, 4, 6 h	X	X	X
ECG (12 lead)			X									
Bilateral MRI[10]			X				X				X	X
Strength (QMT – by fixed system)[11]	X	X	X				X				X	X
Strength (QMT – by handheld device)[11]	X	X	X		X	X	X	X	X		X	X
Lower Leg Functional Assessments[12]	X	X	X			X	X	X	X		X	X
Upper Arm Functional Assessments[13]	X	X	X			X	X	X	X		X	X
FSHD-Health Index	X	X	X			X	X	X	X		X	X
Monitoring of concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X
Monitoring of adverse events			X	X	X	X	X	X	X	X	X	X
Study drug administration[14]			X			X	X	X	X			

[1] Study procedures must be done prior to administration of study drug. Reference the Study Manual for the order of study procedures. Laboratory samples and functional/strength assessments may be collected up to 24 hours prior to administration of study drug.
[2] Patients who discontinue prior to the Day 106/ET visit should complete the Day 106/ET visit at the time of discontinuation and return for the remaining follow-up period visit. If an MRI has been completed within 4 weeks of discontinuation, it does not need to be repeated as part of the Day 106/ET visit procedures.
[3] Pregnancy test for women of childbearing potential to be performed and negative result confirmed before dosing.
[4] Full physical examination (skin, head, eyes, ears, nose, throat and neck, lymph nodes, cardiovascular, respiratory, gastrointestinal, musculoskeletal, and neurological) at screening and Day 106/ET; limited physical examination (skin, cardiovascular, respiratory, musculoskeletal, and neurological assessments) for Days 1, 22, 43, 64, 85.
[5] Injection site examination including but not limited to evaluating erythema, pain, bruising, bleeding, and other signs of discomfort or skin reaction to be completed at least 30 minutes after injection.
[6] Vital signs (weight, heart rate, systolic and diastolic blood pressure) must be taken prior to administration of study drug on dosing days. Height is collected only at screening.
[7] Tests defined in Appendix 2, Table 7.
[8] If a patient has a positive ADA result at Day 141, the patient will be asked to return to the clinical site for additional follow-up approximately every 3 months until a negative or stable result is obtained.
[9] PK samples on dosing day have a ±15 minute window for post-dose sample collection. Pre-dose samples may be collected up to 4 hours prior to dosing. Day 2 and 86 sample collection should be 24 hours post dose ±3 hours.
[10] MRI assessments should be completed within 5 days prior to the scheduled dose administration. MRI assessments during the follow up period (Day 106/ET and Day 141) have a ± 5 day window.
[11] Maximum voluntary isometric contraction testing (MVICT) of both TA and BB muscles will be conducted for all cohorts at screening visits and Day 141 only using a handheld dynamometer, only the muscle under study will be tested (i.e., TA or BB). Both sides will be tested for all visits (right and left).
[12] TA cohort only: 10-meter walk/run, 4-stair climb, 6-minute walk test, and gait analysis; lower leg assessments will be collected for the BB cohorts at screening visits and Day 141 only.
[13] BB cohort only: PUL testing (middle domain) testing; PUL testing (middle domain) will also be performed on patients in TA cohorts at screening visits and Day 141 only. Non-ambulatory patients in the BB cohort can opt out of the TA screening tests.
[14] Study drug administration should occur within 21 days (± 3 days) of the previous dose.
[15] All visit day windows should be considered relative to the date of the previous dose of study drug. Actual visit days may be different than planned (e.g., Day 8, Day 22) due to windows on visits and potential dosing delays.
[16] Genetic testing for FSHD1 to be performed at screening if patient has not already had testing performed or previous results cannot be used to determine eligibility.

Part 2

	Screening		Double-Blind, Placebo-Controlled												Open-Label			ET[17]	EOS[18]
Cycle(s)	–	–	1			2	3	4	5		6	7	8	9	10, 15	16	11, 12, 13; 14, 17	--	–
Planned Day(s)	-28 to -21	-7 (±3d)	1[1]	2	8 (±1d)[2]	22[1] (±1d)[2]	43[1] (±3d)[2]	64[1] (±3d)[2]	85[1] (±3d)[2]	86	106[1] (±3d)[2]	127[1] (±3d)[2]	148[1] (±3d)[2]	169[1] (±3d)[2]	(190, 295)[1] (±3d)[2]	316[1] (±3d)[2]	(211, 232, 253; 274, 337)[1] (±3d)[2]	358 (±3d)[2]	393 (±3d)[2]
Informed consent	X																		
Inclusion/exclusion criteria	X																		
Urine pregnancy test[3]			X			X	X	X	X		X	X	X	X	X	X	X		
Medical history	X	X	X																
Genetic testing[4]	X																		
Full physical examination[5]	X										X				X			X	X
Limited physical examination[6]			X			X	X	X	X			X	X	X		X	X		
Injection site examination[7]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Vital signs[8]	X	X	X		X	X	X	X	X		X	X	X	X	X	X	X	X	
Hematology[9]	X		X			X	X	X	X		X		X		X		X	X	
Chemistry[9]	X		X			X	X	X	X		X		X		X		X	X	
Urinalysis[9]	X		X				X	X	X		X		X		X		X	X	
PD Biomarkers	X		X			X	X	X	X		X		X		X		X	X	X
Anti-drug antibody			X		X	X	X	X	X		X		X		X		X	X	X[18]
Serum PK[10]			0, 2, 4, 6 h	X	X				0, 2, 4, 6 h	X					X			X	X

	Screening		Double-Blind, Placebo-Controlled												Open-Label			ET[17]	EOS[18]
Cycle(s)	–	–	1			2	3	4	5		6	7	8	9	10, 15	16	11, 12, 13; 14, 17	--	–
Planned Day(s)	-28 to -21	-7 (±3d)	1[1]	2	8 (±1d)[2]	22[1] (±1d)[2]	43[1] (±3d)[2]	64[1] (±3d)[2]	85[1] (±3d)[2]	86	106[1] (±3d)[2]	127[1] (±3d)[2]	148[1] (±3d)[2]	169[1] (±3d)[2]	(190, 295)[1] (±3d)[2]	316[1] (±3d)[2]	(211, 232, 253; 274, 337)[1] (±3d)[2]	358 (±3d)[2]	393 (±3d)[2]
ECG (12 lead)		X		X					4 h[19]	X									
Bilateral MRI[11]			X				X				X				X			X	X
Strength (QMT – by handheld device)[12]	X	X	X		X	X	X	X	X		X		X		X		X	X	X
Lower Leg Functional Assessments[13]	TA & BB	TA & BB	TA			TA	TA	TA	TA		TA		TA	TA	TA & BB Day 190 TA Day 295		TA	TA	TA & BB
Upper Arm Functional Assessments[14]	BB & TA	BB & TA	BB			BB	BB	BB	BB		BB		BB	BB	BB & TA Day 190 BB Day 295		BB	BB	BB & TA
FSHD-Health Index	X	X	X			X	X	X	X		X		X		X		X	X	X
Monitoring of concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Monitoring of adverse events			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Randomization[15]			X																
Study drug administration[16]			X			X	X	X	X		X	X	X	X	X	X	X		

	Screening		Double-Blind, Placebo-Controlled												Open-Label			ET[17]	EOS[18]
Cycle(s)	–	–	1			2	3	4	5		6	7	8	9	10, 15	16	11, 12, 13; 14, 17	–	–
Planned Day(s)	-28 to -21	-7 (±3d)	1[1]	2	8 (±1d)[2]	22[1] (±1d)[2]	43[1] (±3d)[2]	64[1] (±3d)[2]	85[1] (±3d)[2]	86	106[1] (±3d)[2]	127[1] (±3d)[2]	148[1] (±3d)[2]	169[1] (±3d)[2]	(190, 295)[3] (±3d)[2]	316[1] (±3d)[2]	(211, 232, 253; 274, 337)[1] (±3d)[2]	358 (±3d)[2]	393 (±3d)[2]
MMT assessment (MRC)	X														X			X	X

[1] Study procedures must be done prior to administration of study drug. Reference the Study Manual for the order of study procedures. Laboratory samples and functional/strength assessments may be collected up to 24 hours prior to administration of study drug. Time of study drug administration is the time of the first injection. The Day 1 visit is timed relative to the Screening Day -28 to Day -21 visit. The Day -7 visit is timed relative to the scheduled Day 1 visit.

[2] All visit day windows should be considered relative to the date of the previous dose of study drug. Actual visit days may be different than planned (e.g., Day 8, Day 22) due to windows on visits and potential dosing delays.

[3] Pregnancy test for women of childbearing potential to be performed and negative result confirmed before dosing.

[4] Genetic testing for FSHD1/FSHD2 to be performed at screening if patient has not already had testing performed or previous results cannot be used to determine eligibility. Genetic testing for FSHD1/FSHD2 to be performed during the study if first degree relative is used for study eligibility but patient has not had testing previously performed or if patient's genetic documentation is inadequate with respect to FSHD1 D4Z4 fragment size or repeat number

[5] Full physical examination includes skin, head, eyes, ears, nose, throat and neck, lymph nodes, cardiovascular, respiratory, gastrointestinal, musculoskeletal, and neurological

[6] Limited physical examination includes skin, cardiovascular, respiratory, musculoskeletal and neurological assessments

[7] Injection site examination including but not limited to evaluating erythema, pain, bruising, bleeding, and other signs of discomfort or skin reaction to be completed at least 30 minutes after injection.

[8] Vital signs (weight, heart rate, systolic and diastolic blood pressure) must be taken prior to administration of study drug on dosing days. Height is collected only at screening.

[9] Tests defined in Appendix 2, Table 7.

[10] PK samples on a dosing day have a ±15 minute window for post-dose sample collection, based on the time of the first injection. Pre-dose samples may be collected up to 4 hours prior to dosing. Day 2 and 86 sample collection should be 24 hours post dose ±3 hours.

[11] MRI assessments should be completed within 5 days prior of the scheduled dose administration. MRI assessments during the follow up period (Day 358/ET and Day 393/EOS) have a ± 5 day window

[12] Maximum voluntary isometric contraction testing (MVICT) of both TA and BB muscles will be conducted for all cohorts at screening visits, Day 190 and Day 393/EOS At all other visits, only the muscle under study will be tested (i.e., TA or BB). Both sides will be tested for all visits (right and left).

[13] TA cohort only: 10-meter walk/run, 4-stair climb, 6-minute walk test, and gait analysis are performed at all visits: 100-meter timed test begins at Day 169 and continues throughout open-label visits; lower leg assessments will be collected for the BB cohorts at screening visits, Day 190 and Day 393/EOS only.

[14] BB cohort only: PUL mid-level/elbow dimension performed at all visits; additional PUL high level/shoulder dimension begins at Day 169 and continues throughout open-label visits; PUL testing will also be performed on patients in TA cohorts at screening visits (mid level), Day 190 (high and mid-level) and Day 393/EOS (high and mid-level) only.
Non-ambulatory patients in the BB cohort can opt out of the TA assessments.

[15] Randomization should occur within 24 hours prior to Day 1 dose.

[16] Study drug administration should occur within 21 days (± 3 days) of the last dose.

[17] Patients who discontinue prior to the Day 358/ET visit should complete the Day 358/ET visit at the time of discontinuation and return for the remaining follow-up period visit. If an MRI has been completed within 4 weeks of discontinuation, it does not need to be repeated as part of the Day 358/ET visit procedures.

[18] If a patient has a positive ADA result at Day 393/EOS, the patient will be asked to return to the clinical site for additional follow-up approximately every 3 months until a negative or stable result is obtained.

[19] ECG is to be conducted ±1 hour of the 4h PK sample.