Protocol C3391001

A PHASE 1B MULTICENTER, OPEN-LABEL, SINGLE ASCENDING DOSE STUDY TO EVALUATE THE SAFETY AND TOLERABILITY OF FORDADISTROGENE MOVAPARVOVEC (PF-06939926) IN AMBULATORY AND NON-AMBULATORY SUBJECTS WITH DUCHENNE MUSCULAR DYSTROPHY

Statistical Analysis Plan (SAP)

Version: 6

Date: 27-Oct-2022

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APPENDICES

1. VERSION HISTORY

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
6 27-Oct-2022	Amendment 9 7-Mar-2022	Analysis of study endpoints utilizing external controls will be described separately in a supplementary SAP. SAP content clarification. Updated the analysis description related to the sirolimus cohort given the sample size.	Throughout the SAP: Removed content related to synthetic controls (or external controls) including Section 4, Section 5.1, Section 5.3.5, Section 5.4, Section 6.3.2.1, Section 6.3.4.1, Appendix 2 and methods of deriving the external control sections, related reference articles.
			Section 2.1, 2.2, 2.4.1: Consolidated description of study objectives, endpoints, and estimands into a single table. Removed estimand for safety, immune response, viral vector shedding, and the sirolimus evaluation endpoints.
			Sections 2.1, 3.2.1.2, and 6.2.2: Added endpoints and analyses for the newly developed immunofluorescence assay mini-dystrophin specific antibody.
			Sections 2.1, 3.2.1.1, and 6.2.1: Removed Western Blot assay endpoints and analyses and clarified endpoint definitions.
			Section 2.5: Streamlined the study design description to reflect the current design and enrollment.
			Throughout the SAP: Clarified that since only one participant received sirolimus, no summaries will be provided for the sirolimus cohort.
			Section 3.1.5: Added LV fractional shortening and LV mass index to the cardiac MRI endpoints.
			Section 3.3.1: Added specific immunogenicity endpoints.
			Sections 3.3.3 and 6.3.5: Clarified endpoint definitions and revised definitions of time to clearance of viral vector shedding endpoints to first identify the first set of 2 consecutive negative results and time to clearance is defined as (1) time to first of 2 consecutive negative results and (2) time to last of 2 consecutive negative results.
			Sections 4.2, 4.2.1, and 4.2.2: Removed reference to exclusions due to major protocol deviations to align with analysis sets.
			Section 5.1: Rather than performing statistical tests at the two-sided α=0.05 level, nominal p-values will be presented since no hypothesis testing is to be performed.
			Section 5.3.2: Revised to indicate that values below the LLOQ will not be imputed.
			Sections 5.3.3: Removed sensitivity analysis and revised to impute values below the LLOQ with 0.5*LLOQ.

			Sections 5.3.4: Revised to impute values below the LLOQ or "undetectable" as 0.5*LLOQ for graphical presentations but not impute for summary statistics. For values above the ULOQ, missing values will be imputed as ULOQ for graphical displays and summary statistics.
			Section 5.3.5 and Appendix 2: Revised to include the worst value if multiple values are observed within a window.
			Section 5.3.6 and 6.3.2.2: Moved all analyses to assess the impact of the COVID-19 pandemic to a supplementary SAP.
			Section 6.1.2. Clarified that if there is a local and central laboratory collection on the same day, the central laboratory value will be used for analysis. Removed summary of change from baseline to last observation in laboratory tests. Clarified that externally normalized values will be normalized to pediatric textbook ranges. Added content related to cTn-I analyses.
			Section 6.1.3: Added categorical tables for the first 7 days of follow-up and the first 30 days of follow-up.
			Section 6.1.4: Removed listing.
			Section 6.1.5: Removed summaries of physical exam and neurological exam since any significant findings are recorded as AEs.
			Section 6.3.2.1: Added a sentence to set the total score to missing if one or more of the 17 items of NSAA is missing. Clarified the definition of skills gained and added definitions for rise from floor velocity and 10 meter run/walk velocity.
			Section 6.4.2: Added age subgroups
			Section 6.5.1: Removed ventilatory support.
			Section 6.4.3: Removed reference to flagging specific mutations in the listing.
			Section 8: Moved references to endnotes.
			Throughout the SAP: Changed PF-06939926 to fordadistrogene movaparvovec.
			Throughout SAP: Removed refence to sponsor reporting standards.

2. INTRODUCTION

The investigational product, fordadistrogene movaparvovec (PF-06939926), is a gene therapy viral construct that consists of a recombinant AAV9 vector with a miniaturized version of the DMD gene which encodes the domains minimally required for functionality of the dystrophin protein. Cardiac and skeletal muscle-specific transgene expression is mediated by the synthetic promoter, hCK. This agent is in development for the treatment of DMD, an X-linked neuromuscular disease primarily affecting boys.

Protocol C3391001 is the FIH/FIP study with fordadistrogene movaparvovec. This study aims to evaluate the safety and tolerability following a single dose of fordadistrogene movaparvovec in ambulatory and non-ambulatory participants with DMD. Other objectives include pharmacodynamics of dystrophin expression and distribution, assessments relevant to muscle quality and function and dose selection for future clinical development.

This SAP provides the detailed methodology for summary and statistical analyses of the data collected in study C3391001. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition or its analysis will also be reflected in a protocol amendment.

2.1. Study Objectives, Endpoints, and Estimands

Objectives	Endpoints	Estimands
Primary Objectives:	**Primary Endpoints:**	**Primary Estimands:**
To determine the safety and tolerability of a single IV infusion of fordadistrogene movaparvovec in participants with DMD at ascending doses.	Incidence of both dose-limiting and treatment-related AEs, inclusive of infusion and injection site reactions, through 1 year post-treatment.	Not applicable
	Incidence, severity, and causal relationship of TEAEs through 1 year post-treatment.	
	Incidence and magnitude of abnormal laboratory findings through 1 year post-treatment.	
	Abnormal and clinically relevant changes through 1 year post-treatment in: • Physical examination; • Neurological examination; • Weight; • Vital signs; • ECG; • Cardiac MRI- (or echocardiogram-) measured LVEF; • C-SSRS	

Objectives	Endpoints	Estimands
Secondary Objectives:	**Secondary Endpoints:**	**Secondary Estimands:**
1) To determine the amount and distribution of mini-dystrophin expression in the muscle of participants with DMD following a single IV infusion of fordadistrogene movaparvovec at ascending doses.	Evidence of mini-dystrophin expression and transduction in muscle by immunohistochemistry and LC-MS using muscle biopsies at baseline, 2 or 3 and 6 or 12 months post-treatment	Change from Baseline at early timepoints (2 or 3 months) and later timepoints (12 months; 6-month may be used if 12-month information is not available) in the following measures: • Mini-dystrophin levels, expressed as fmol/mg of total protein, measured by LC-MS assay, LEMPSSLMLEVPTHR (LEMP) peptide • Total dystrophin levels, expressed as fmol/mg of total protein, measured by LC-MS assay, LLQVAVEDR (LLQV) peptide • Total dystrophin levels, expressed as percent normal, measured by LC-MS assay, LLQVAVEDR (LLQV) peptide • Percent of dystrophin and mini-dystrophin positive muscle fibers derived from the individual fiber MSD data, measured by immunofluorescence assay using DYSB antibody (IF DYSB) • Percent of mini-dystrophin positive muscle fibers derived from the individual fibers MSD data, measured by immunofluorescence assay using mini-dystrophin specific antibody (IF mini-dys)
2) To evaluate long term safety of a single IV infusion of fordadistrogene movaparvovec in participants with DMD at ascending doses.	Incidence, severity and causal relationship of TEAEs and clinically significant safety findings, as described for the primary endpoints (above) through 5 years post-treatment.	Not applicable.
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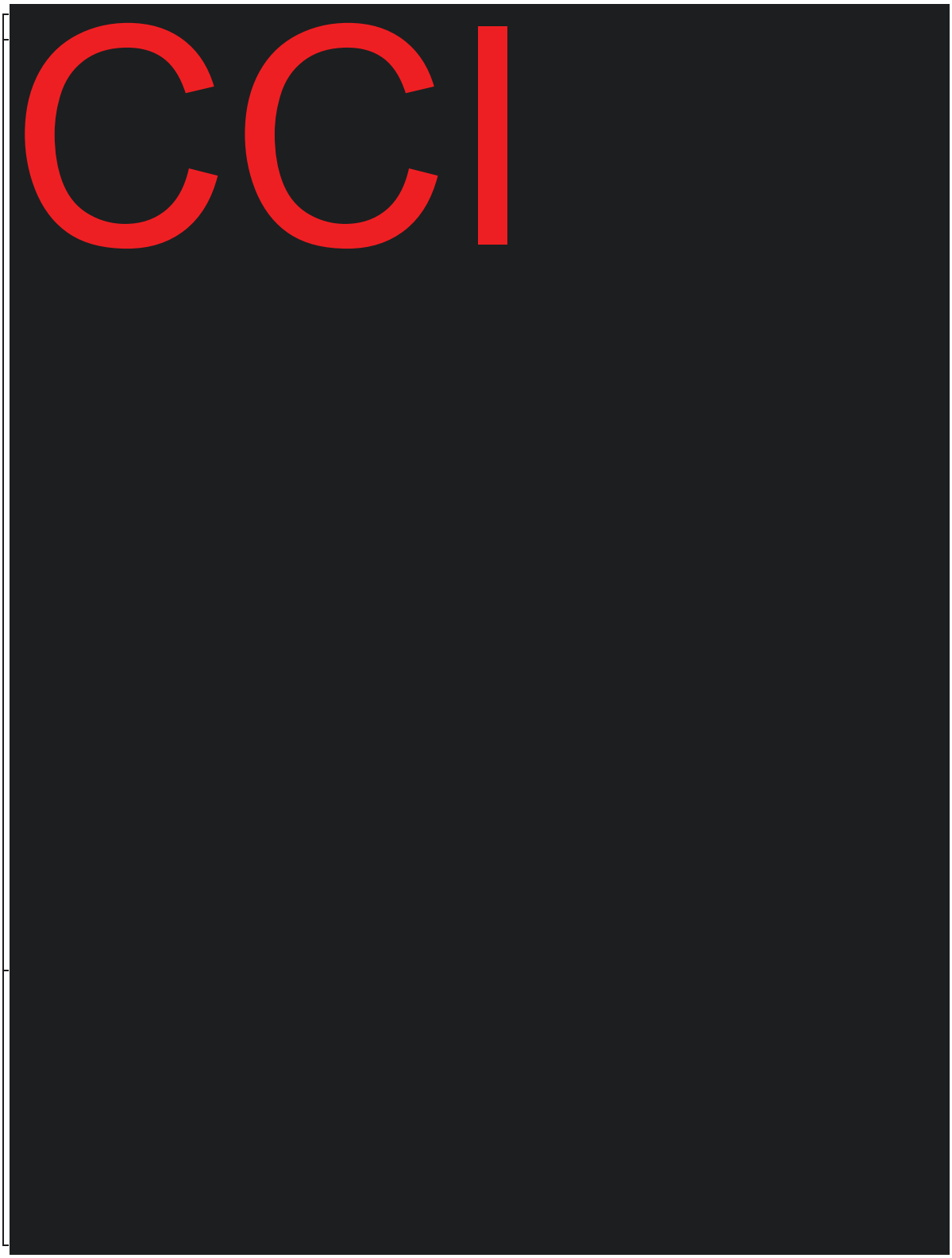
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A description of an estimand includes 4 attributes to define the treatment effect of interest. The following subsections provide the details of the estimands linked to the specific objectives and endpoints when applicable.

2.2. Primary Estimand(s)

Not applicable.

2.3. Secondary Estimand(s)

Analyses of the secondary endpoints by population (ambulatory, non-ambulatory, overall) and by dose cohort are described in Section 6.2. For each secondary endpoint, the four attributes associated with the estimand are summarized below.

- Population: Among ambulatory and non-ambulatory participants who meet the inclusion and exclusion criteria, those who receive a single IV infusion of fordadistrogene movaparvovec.

- Variable: Secondary endpoints listed in Section 2.1.

- Intervention effect: Effect of treatment on dystrophin/mini-dystrophin expression and distribution through 1 year post-treatment, regardless of adherence to background and protocol-mandated glucocorticoid regimens.

- Population-level summary: Changes from Baseline (pre- and post-) for treatment difference through 1 year post-treatment

2.5. Study Design

This FIH/FIP study is an open-label, multi-center, ascending dose study designed to evaluate the safety and tolerability of a single IV infusion of fordadistrogene movaparvovec in

participants with DMD. There are 22 participants who received a single dose of fordadistrogene movaparvovec between 1E14 vg/kg and 3E14 vg/kg per the ITR-based method or 2E14 vg/kg per the TG-titer method.

Fordadistrogene movaparvovec at 3E14 vg/kg per the ITR-based titer method is approximately equivalent to 2E14 vg/kg per the TG-titer method. Therefore, participants dosed at 3E14 vg/kg per the ITR-based titer method or at 2E14 vg/kg per the TG-titer method will be grouped in the high dose cohort.

1. Ambulatory low dose cohort (N=3): 1E14 vg/kg based on the ITR method

2. Ambulatory high dose cohort (N=16): 3E14 vg/kg based on the ITR method or 2E14 vg/kg based on the TG method

3. Non-ambulatory high dose cohort (N=3): 2E14 vg/kg based on the TG method

    - Sirolimus non-ambulatory high dose cohort (N=1): 2E14 vg/kg per the TG-based titer method.

Note that a safety profile will be provided for the one participant who received sirolimus. No other summaries will be provided since only one participant received sirolimus.

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS

3.1. Primary Endpoints

- *Incidence of both dose-limiting and treatment-related AEs, inclusive of infusion and injection site reactions, through 1 year post-treatment.*

- *Incidence, severity, and causal relationship of TEAEs through 1 year posttreatment.*

- *Incidence and magnitude of abnormal laboratory findings through 1 year posttreatment.*

- *Abnormal and clinically relevant changes in physical and neurological examinations, weight, vital signs, ECG, cardiac MRI (or echocardiogram-) measured LVEF, CSSRS through 1 year posttreatment.*

3.1.1. Adverse Events

All nonserious AEs and SAEs occurring in a participant during the active collection period, which begins after obtaining informed consent will be recorded on the AE section of the CRF. MedDRA will be used to classify all AEs with respect to SOC and PT.

Relative to fordadistrogene movaparvovec administration:

Any AE that starts following start of treatment of fordadistrogene movaparvovec will be counted as treatment-emergent.

Events that occur in a non-treatment period (applies to screening until study drug administration only) will not be counted as treatment-emergent as this is a single period study.

Relative to sirolimus administration: Any AE that starts following start of treatment of sirolimus will be counted as treatment-emergent.

3.1.2. Laboratory Data

Safety laboratory tests will be performed as described in the protocol. To determine if there are any clinically significant laboratory abnormalities, the hematological, clinical chemistry (serum) and urinalysis safety tests will be assessed against abnormality criteria. Moreover, GLDH will be summarized to monitor the liver toxicity. Other parameters to be summarized can be found in Section 6.1.2.

Baseline is defined as the last pre-dose measurement.

3.1.3. Vital Signs

Blood pressure (systolic and diastolic), respiratory rate, and pulse rate measurements will be taken at times detailed in the Schedule of Activities given in the protocol. Body temperature may also be collected if clinically indicated.

Baseline is defined as the last pre-dose recording.

3.1.4. Electrocardiograms

Triplicate 12-lead ECGs will be recorded according to the Schedule of Activities in the protocol.

The QT interval, QTcF interval, PR interval, RR interval, QRS complex and heart rate will be recorded at each assessment time.

If not supplied, QTcF interval will be derived using Fridericia's heart rate correction formula: $QTcF = QT / (RR)^{1/3}$ where RR = 60 / heart rate (if not provided)

The average of the triplicate readings will be calculated for each ECG parameter.

Baseline will be defined as the average of the last triplicate ECG measurements collected prior to dosing on Day 1.

3.1.5. Cardiac MRI (or Echocardiogram, if necessary)

Cardiac MRI (or echocardiogram) will be collected at times specified in the Schedule of Activities section of the protocol. The mean absolute and percent change from baseline in

cardiac parameters including LVEF, LV Fractional Shortening (%), LV mass index, will be evaluated and the number (%) of participants with the presence of late gadolinium enhancement will be provided.

Baseline is defined as the last pre-dose measurement.

3.1.6. Other Safety Data

Additional safety data (neurological and physical examination, infusion site reaction and C-SSRS will be collected as described in the protocol and will be listed and/or summarized.

3.2. Secondary Endpoint(s)

3.2.1. Mini-dystrophin expression and distribution

1. Change from Baseline in percent normal dystrophin expression level in biceps brachii muscle biopsies at 2 (or 3) and 12 months post-treatment using an LC-MS assay

2. Change from Baseline in percent of dystrophin-positive muscle fibers in biceps brachii muscle biopsies at 2 (or 3) and 12 months post-treatment as assessed by immunofluorescence.

3.2.1.1. Mini-dystrophin expression

Dystrophin and mini-dystrophin expression levels will be measured by LC-MS assay. For the LC-MS assay, there are two reportable peptides:

1. LEMPSSLMLEVPTHR (LEMP) peptide results will quantify the mini-dystrophin transgene protein levels following treatment with fordadistrogene movaparvovec.

   No mini-dystrophin transgene protein will be expressed in the tissue samples obtained prior to the administration of fordadistrogene movaparvovec.

2. LLQVAVEDR (LLQV) peptide results will quantify the total dystrophin protein including both full-length, endogenous dystrophin protein and the mini-dystrophin transgene protein.

   Revertant endogenous fibers usually exist due to a mechanism that restores the reading frame (Klein et al., 1992[1]). Dystrophin levels from revertant fibers may be measurable by LC-MS assay. Baseline is defined as the endogenous dystrophin levels reflected in the tissue sample collected prior to the administration of fordadistrogene movaparvovec.

3.2.1.2. Mini-dystrophin distribution

Muscle fibers expressing both endogenous dystrophin protein and mini-dystrophin transgene protein will be identified by positive staining. Percentage of positive muscle fibers will be calculated for each muscle biopsy.

Dystrophin distribution will be evaluated by immunofluorescent staining using two antibodies.

1. DYSB antibody that recognizes both endogenous dystrophin protein and mini-dystrophin transgene protein from the pre-dose tissue biopsy for the endogenous dystrophin antibody.

   For the baseline assessment, the distribution of the fiber MSD for each individual fiber will be evaluated for each participant to assess the expression level of total dystrophin protein. Baseline will be derived from the fiber MSD for each individual fiber in the tissue biopsies that were obtained prior to the administration of fordadistrogene movaparvovec.

2. Mini-dystrophin specific antibody that only recognizes the mini-dystrophin transgene protein

   No mini-dystrophin transgene protein fibers will be contained in the tissue samples obtained prior to the administration of fordadistrogene movaparvovec.

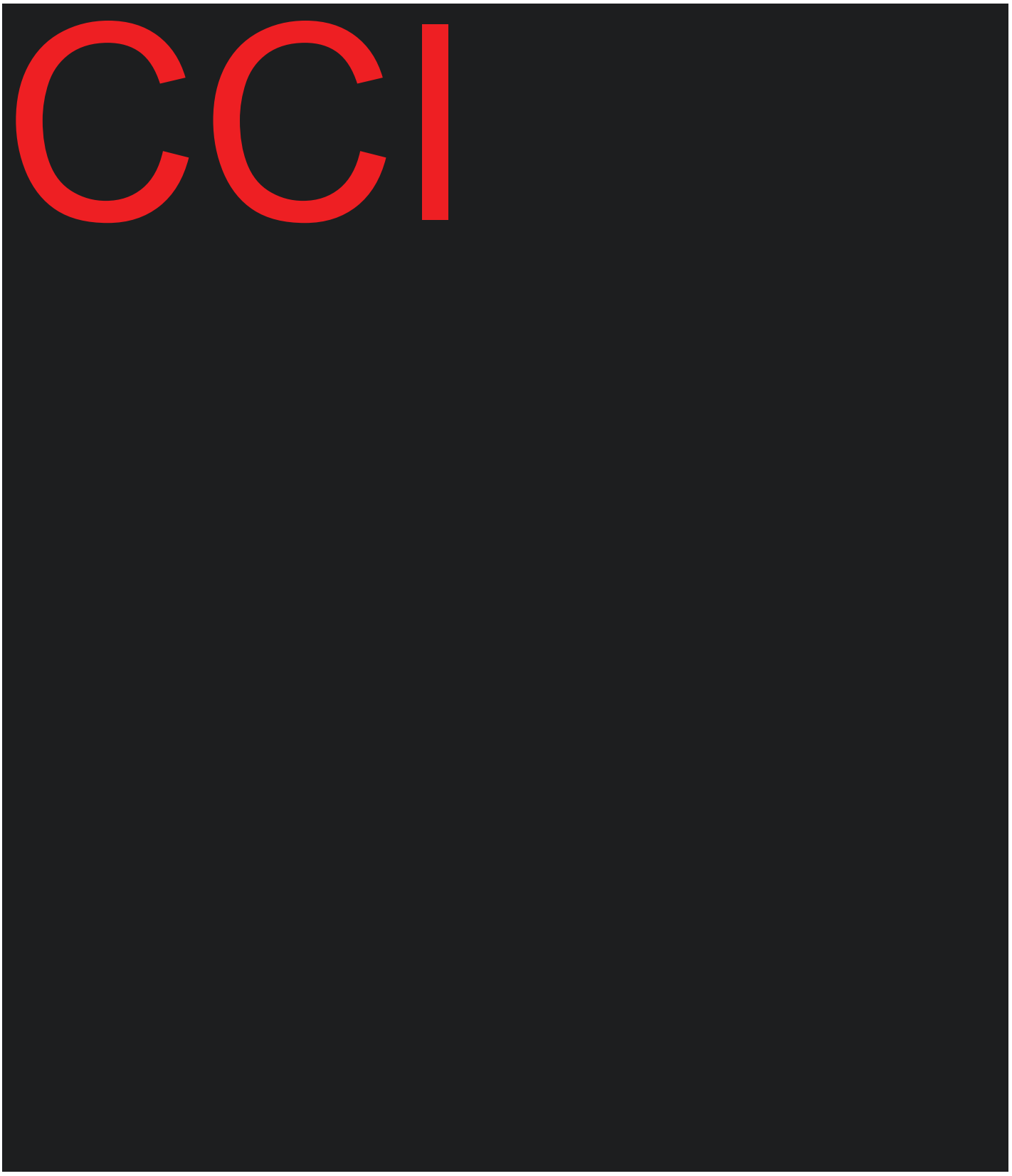
The positive threshold value for the mini-dystrophin specific antibody represents a pooled average MFI value from 19 ambulatory baseline, pretreatment biopsies.

For the determination of percent of positive muscle fibers and the derivation of the baseline value, refer to Section 6.2.2 for details.

3.2.2. Long-term safety

Incidence, severity and causal relationship of TEAEs and clinically significant safety findings, as described for the primary endpoints (above) through 5 years post-treatment.

Baseline is defined the same as those described in Section 3.1.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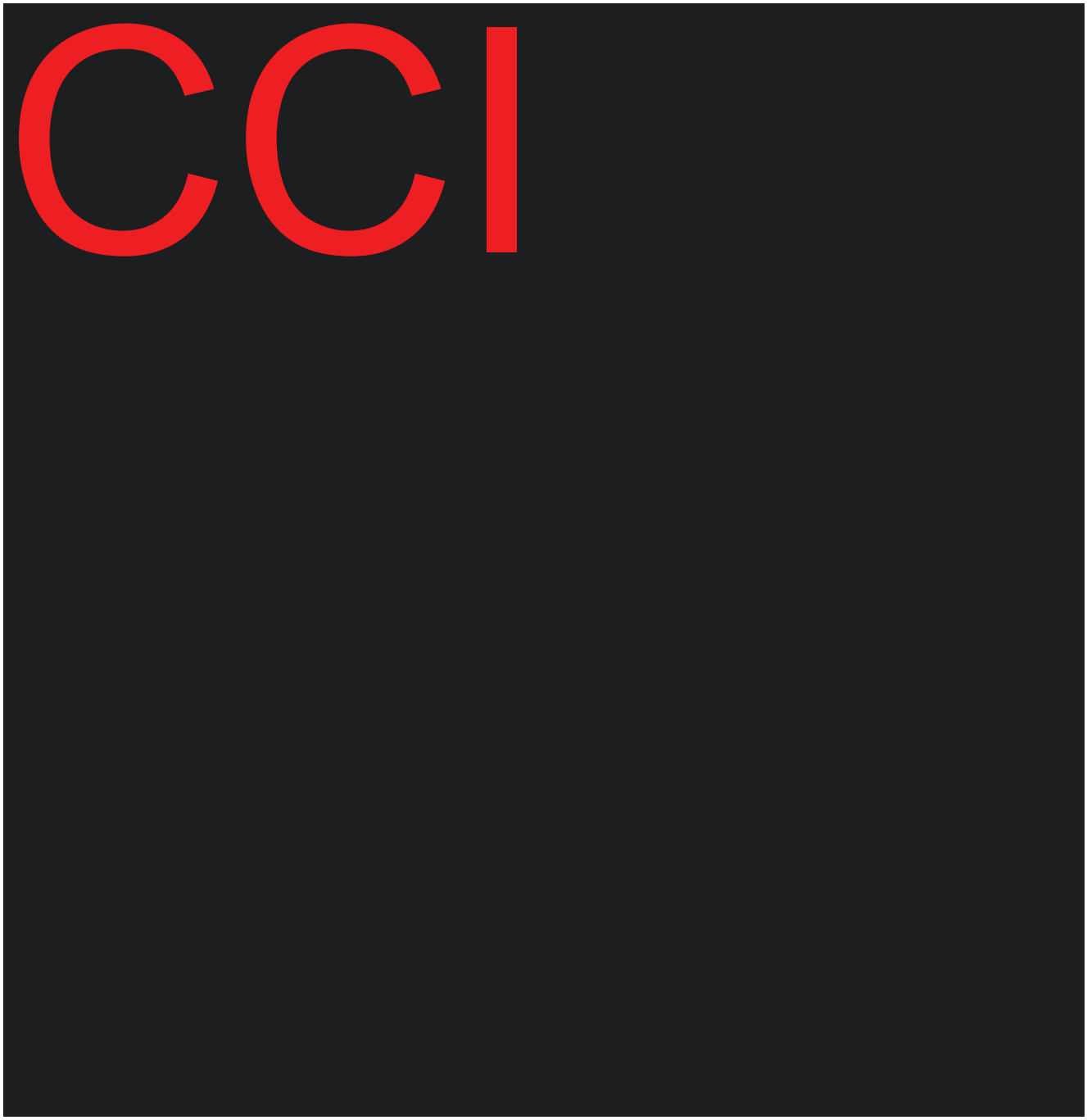

3.4. Baseline Variables

Participants' demographics and medical history will be collected at screening visit.

The ambulatory status upon enrollment, ie, whether the participant is able to walk unassisted and without braces for at least 10 meters, is considered as a stratification factor. All analyses will be conducted by the ambulatory status at baseline unless otherwise specified.

Screening age (years) covariate is defined as the participant's age on the day of the Screening visit. Age at dosing (years) covariate is defined as the participant's age on the day of the administration of fordadistrogene movaparvovec (Dosing visit).

The following baseline variables will be summarized:

- Demographic characteristics including age, race, and ethnicity, where age is calculated as age in years at the Screening visit and at the Dosing visit.
- Physical measurements at Baseline include height (cm) and weight (kg) where Baseline is defined as the last non-missing pre-dose measurement. For non-ambulatory participants, the height may represent the estimated height derived from the participant's ulnar length per protocol's instructions if the participant is unable to stand safely.
- Background glucocorticoid regimen (including specific medication and daily dose in mg/kg, and duration of current glucocorticoid regimen use) at the Screening visit, and age at initiation of any glucocorticoid use (years).
- General medical history, including diseases or syndromes that are ongoing ('present') at, or stopped ('past') before, Screening. MedDRA will be used to code the disease/syndrome.

4. ANALYSIS SETS (POPULATIONS FOR ANALYSIS)

Data for all participants will be assessed to determine if the participants meet the criteria for inclusion in each analysis population prior to releasing the database.

For purposes of analysis, the following populations are defined:

Population	Description
Enrolled	All subjects who sign the informed consent document.
FAS	All enrolled participants who receive fordadistrogene movaparvovec. Analyses will be performed on the FAS population unless otherwise specified.
Safety	All enrolled participants who receive fordadistrogene movaparvovec. Participants will be analyzed according to the intervention they actually received.
Pharmacodynamic Analysis Set	All participants in the FAS who have baseline and at least one post-dose mini-dystrophin parameters of interest reported.

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Population	Description
Ambulatory	All participants in the FAS who meet the study eligibility criteria for ambulatory status at enrollment: able to walk 10 meters unassisted and rise from floor within seven seconds.
Non-ambulatory	All participants in the FAS who meet the study eligibility criteria for non-ambulatory status at enrollment: unable to walk 10 meters unassisted.
Low dose cohort	All participants in the FAS who receive 1E14 vg/kg per the ITR-based method.
High dose cohort	All participants in the FAS who receive 3E14 vg/kg per the ITR-based method or 2E14 vg/kg per the TG-based titer method.
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Non-sirolimus cohort	All participants in the FAS who do not receive sirolimus within 2 weeks before and after fordadistrogene movaparvovec infusion.
Regardless of adherence to glucocorticoid regimen	All participants in the FAS, regardless of adherence to background or protocol-mandated glucocorticoid regimens.

The term “ambulatory status at baseline” will be used to describe the stratification factor (ambulatory versus non-ambulatory) used to partition the study participants and report the corresponding group results. The term “overall” will be used to refer to all participants in the FAS.

4.1. Treatment Misallocations

All analyses will be performed on an “as-treated” basis and will not include data from participants who are enrolled but not treated.

If a participant takes a treatment that is not consistent with the assigned treatment, for example, takes a dose level inconsistent with the assigned cohort, then the participant will be reported under the treatment label that he actually received for all analyses, where applicable. A separate listing will be compiled to indicate the actual dose level and the nominal dose received, where applicable.

4.2. Protocol Deviations

A full list of protocol deviations will be compiled and reviewed to identify major and minor deviations prior to database closure.

5. GENERAL METHODOLOGY AND CONVENTIONS

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CCI The primary analysis of the entire study will be performed at study participant data set release after the last participant completing Week 52 visit. Secondary safety analyses will be performed on the long term follow up data through 5 years post-treatment. For the analyses of a subgroup with <3 participants, individual participant profile descriptive analyses will be conducted instead of summary statistics across the subgroup.

5.1. Hypotheses and Decision Rules

There is no formal hypothesis testing in this study according to the original design of this FIH study. Because the study has a relatively small sample size and is not designed to have sufficient power to detect treatment differences, it is expected that hypothesis testing is not likely to reach statistical significance unless there is dramatic treatment effect. Nominal p-values will be presented. The 95% CI for the change from baseline in secondary (dystrophin expression/distribution)CCI functional endpoints will be estimated.

5.2. General Methods

Bootstrap to estimate the parameter of interest

Given the relatively small sample size of this study and the variability of the various outcome parameters, the non-parametric bootstrapping method (Efron 1979[2], Efron 1993[3]) will be used to compute the 100(1-α) % CIs of the parameters of interest for robust evaluation without underlying distributional assumptions. Specifically, the sampling distributions of the statistics derived from 10,000 bootstrap samples such as the mean of the change from baseline in an endpoint will be used to construct the 95% CI of the mean when $\alpha = 0.05$.

5.2.1. Analyses for Continuous Endpoints

For continuous variables, the data will be summarized using the number of participants, mean, median, SD, minimum, maximum.

5.2.2. Analyses for Categorical Endpoints

For categorical or ordinal variables, number of participants, numbers and percentages of participants meeting the categorical criteria will be supplied.

5.2.3. Analyses for Time-to-Event Endpoints

Time-to-event endpoints will be summarized using the Kaplan-Meier method (Kaplan and Meier 1958[4]) and estimated time-to-event-free curves will be displayed graphically when appropriate. Graphs will describe the number of participants at risk over time. The median, quartiles, and probabilities of an event at particular points in time will be estimated by the

Kaplan-Meier method. CIs for medians and quartiles are based on the Brookmeyer-Crowley method. CIs for the estimated probability of an event at a particular time point will be generated using the Greenwood formula.

5.3. Methods to Manage Missing Data

In listings, values below the LLOQ will be reported as "<LLOQ", where LLOQ will be replaced with the value for LLOQ for the corresponding assay.

5.3.1. Safety endpoints

For the analysis of safety endpoints, the sponsor data standard rules for imputation will be applied.

5.3.2. Immunogenicity endpoints

For immunogenicity assay results including ADA titers, NAb titers, and ELISpot levels, values below the LLOQ will not be imputed for summary statistics.

5.3.3. Secondary pharmacodynamic endpoints

Treatment induced mini-dystrophin levels below the LLOQ quantified by LC-MS assay (LEMP peptide or LLQV peptide) will be imputed as 0.5*LLOQ for summary statistics and graphical presentation.

5.3.6. Missed visits or Assessments out of Protocol-specified Visit Windows

To minimize missing data, unplanned measurements will be included in analyses unless otherwise specified. For summary analyses at scheduled visits, extended time windows for measurements specified in Appendix 2 will be implemented in analysis. If multiple values are observed within a time window, the value closest to the target visit day from the administration of fordadistrogene movaparvovec will be included in the analysis unless specified otherwise. Day 1 is defined as the study drug dosing visit.

6. ANALYSES AND SUMMARIES

6.1. Primary Endpoints

The safety and other endpoints detailed in Section 3.1 will be listed and summarized, where the resulting data presentations will consist of participants from the Safety Analysis Set (as defined in Section 4).

Primary safety analyses will be based on the data through 1-year post-treatment of fordadistrogene movaparvovec. Secondary safety analyses will be based on the long-term follow up data through 5-years post-treatment of fordadistrogene movaparvovec. Adverse events, ECGs, vital signs, physical and neurological examinations, cardiac MRI (or echocardiogram) and safety laboratory data will be reviewed and summarized on an ongoing basis during the study to evaluate the safety of participants. Safety data will be presented in tabular and/or graphical format and summarized descriptively, where appropriate. Analysis windows for these summaries and graphical displays by visit are provided in Appendix 2.

All analyses will be conducted by dose cohort and ambulatory status at baseline to evaluate the safety and tolerability of administering fordadistrogene movaparvovec.

6.1.1. Adverse Events

Incidence and severity of TEAEs will be reported.

Deaths and SAEs will be listed. The following will be summarized by dose cohort and ambulatory status at baseline:

- Incidence and severity of TEAEs (all causalities and treatment-related)
- Withdrawals due to TEAEs; and
- Incidence of SAEs (all causalities and treatment-related).

To evaluate the safety of sirolimus:

The incidence and severity of TEAEs (all causalities and treatment-related) relative to the initiation of treatment with sirolimus through 30 days post-last dose of sirolimus (approximately 44 days post-fordadistrogene movaparvovec infusion per sirolimus administration schedule) will be summarized for those participants who received sirolimus (sirolimus cohort) versus those who did not (non-sirolimus cohort).

No summaries will be provided by sirolimus cohort status since only one participant received sirolimus.

6.1.2. Laboratory Data

Central and local laboratory results will be combined for reporting purposes. Baseline is as defined in Section 3.1.2. Laboratory data including hematology, chemistry and urinalysis will be listed. If there is a local and central laboratory collection on the same day, the central laboratory value will be used for analysis.

The following will be summarized by dose cohort and ambulatory status at baseline. To evaluate the safety of sirolimus, the incidence and magnitude of abnormal laboratory findings through 30 days post the last dose of sirolimus administration will be summarized by sirolimus cohort (sirolimus, non-sirolimus) if there are at least 3 participants in each cohort. No summaries will be provided by sirolimus cohort status since only one participant received sirolimus.

- Incidence of laboratory test abnormalities without regard to baseline abnormality, for normal baseline, and for abnormal baseline.

For select laboratory parameters, data will be summarized by visit descriptively in tabular format and displayed graphically as described below.

Parameter	**Presentation**		
	Table: Changes and Percent changes from baseline By visit	**Shift table:** <LLN*, Normal*, >ULN By visit	**Graph, Box-plot:** Changes from baseline By visit
GLDH	X	X	X
CK	X	X	X
C3 and C4	X		X
Platelets	X		X
Creatinine	X		X

*If the normal range is < or ≤ ULN without the LLN, then the category <LLN will not be presented.

The shift tables will use baseline values categorized as < LLN, Normal, and > ULN, with the shifts being shown into the same categories.

For laboratory parameters summarized descriptively, if the raw laboratory values are obtained using different normal ranges over time or across participants, external normalized values that are normalized to pediatric textbook ranges will be used to summarize the results.

To assess the effect of sirolimus on complement activation, the C4 levels and platelet levels will be summarized descriptively and/or graphically by sirolimus cohort if there are at least 3 participants in each cohort as follows:

- Percent change from baseline to nadir through Day 30 post-fordadistrogene movaparvovec infusion

- Summary statistics including the mean, the location, the location shift between the sirolimus cohort and non-sirolimus cohort, and the corresponding 95% CI (when n ≥ 3) over time and at nadir based on the Hodges-Lehmann method (Hodges & Lehmann, 1963[5]; Hodges Jr & Lehmann 1983[6]).

The nadir is defined as the lowest measurement observed between baseline through Day 30 visit post-fordadistrogene movaparvovec infusion.

No summaries will be provided by sirolimus cohort status since only one participant received sirolimus.

cTn-I:

Baseline values will be categorized as Normal (within Normal Range) and above ULN. The shifts in categories over time showing the number and percent of participants in each category will be presented.

The participants will be divided into two categories: those with baseline cTn-I ≤ 99th percentile (ie, ULN) and those with baseline cTn-I > 99th percentile (ie, ULN). For Centaur assay, the cTn-I 99th percentile (ie, ULN) will be 0.03 ng/mL. The LLOQ is 0.03 and values below LLOQ will set to 0.029. For the Beckman assay, the cTn-I 99th percentile (ie, ULN) will be 19.8 ng/L. The LLOQ is 2.3 ng/L and values below LLOQ will be set to 2.29 ng/L.

For participants with baseline cTn-I > 99th percentile (ie, ULN):

a. Count the number of post-baseline visits at which cTn-I was >3X baseline for each participant. Present the number and percentage of participants who had 0, 1, 2, or 3 visits at which cTn-I was >3X baseline.

b. For post-baseline visits with cTn-I >3X baseline, present descriptive summary statistics (mean, SD, median, Q1, Q3, minimum and maximum) for the maximum cTn-I level.

Graphical displays:

- Spaghetti plot including participants with ≥ 2 visit assessments post-baseline that are >3X baseline.

For participants with baseline cTn-I ≤ 99th percentile (ie, ULN),

a. Count the number of post-baseline visits at which cTn-I was >3X 99th percentile (ie, ULN) for each participant. Present the number and percentage of participants who had 0, 1, 2, or 3 visits at which cTn-I was >3X 99th percentile (ie, ULN).

b. For post-baseline visits with cTn-I >3X 99th percentile, present descriptive summary statistics (mean, SD, median, Q1, Q3, minimum and maximum) for the maximum cTn-I level.

Graphical displays:

- Spaghetti plot including participants with ≥ 2 visit assessments post-baseline that are > 99th percentile (ie, ULN).

All visits (ie, planned and unplanned) will be included in these analyses.

Analysis windows for these summaries and graphical displays by visit are provided in Appendix 2.

Hepatotoxicity evaluation:

To assess potential liver injury, the eDISH plot will be used to present the liver safety data (Watkins et al., 2011[7]). Specifically, the peak AST or ALT (x-axis) and peak TBili (y-axis) will be shown as multiples of (x-fold or y-fold) the ULN on log scales displayed for each participant within a specific timeframe (week 52 for primary safety analyses). Four quadrants defined by the two lines corresponding to 3×ULN for peak AST or ALT and 2×ULN for peak TBili will be shown.

A separate listing displaying the following parameters over time will be generated for participants located in the right upper quadrant.

- ALT, AST, ALP, TBili, GGT, GLDH, CK, creatine kinase myocardial b fraction, platelets, Hepatitis A virus antibody and immunoglobulin M antibody, Hepatitis B virus surface antigen, Hepatitis C virus antibody, and Hy's Law criteria.

6.1.3. Vital Signs

Absolute values and changes from baseline in systolic and diastolic blood pressure, respiratory rate and pulse rate will be summarized by dose cohort, ambulatory status at baseline, and study visit. Tables will be paged by parameter. Baseline is as defined in Section 3.1.3.

The maximum increase from baseline for systolic, diastolic blood pressures and pulse rate will be calculated by first subtracting the baseline value from each post-dose measurement to give the change from baseline. The maximum of these values over the respective period will then be selected, except in the case where a participant does not show an increase. In such an instance, the minimum decrease should be taken.

Similarly, the maximum decrease from baseline for systolic, diastolic blood pressures and pulse rate will be determined by selecting the minimum value of the changes from baseline. In cases where a participant does not show a decrease, the minimum increase should be taken.

Maximum increases and decreases in vital signs will be summarized by dose cohort and ambulatory status at baseline.

Categorical tables (including post-baseline values, maximum increases, and maximum decreases) will also be provided for the first 7 days of follow-up and the first 30 days of follow-up.

6.1.4. Electrocardiograms

If more than one ECG is collected at a nominal time post-dose (for example, triplicate ECGs), the mean of the replicate measurements will be used to represent a single observation at that time point.

Absolute values and changes from baseline in QT, heart rate, QTcF, PR, RR and QRS will be summarized by dose cohort, ambulatory status at baseline, and study visit. Tables will be paged by parameter. Baseline is as defined in Section 3.1.4.

The maximum absolute value (post-dose) and the maximum increase from baseline for QT, heart rate, QTcF, PR, and QRS will be determined over all measurements taken post-dose.

The maximum increase from baseline will be calculated by first subtracting the baseline value from each post-dose measurement to give the change from baseline. The maximum of these values over the respective period will then be selected, except in the case where a participant does not show an increase. In such an instance, the minimum decrease should be taken.

Maximum increase from baseline for QT, heart rate, QTcF, PR and QRS will be summarized by dose cohort and ambulatory status.

ECG endpoints and changes from baseline (QTcF, PR and QRS) will also be summarized descriptively by dose cohort and ambulatory status at baseline using categories as defined in Appendix 1 (for QTc these correspond to ICH E14[8]). Numbers and percentages of participants meeting the categorical criteria will be provided. All planned and unplanned measurements will be counted in these categorical summaries. All values meeting the criteria of potential clinical concern will be listed.

For triplicate ECGs, if any of the three individual ECG tracings has a QTcF value ≥500 msec, but the mean of the triplicates is not ≥500 msec, the data from the participant's individual tracing will be described in a safety section of the CSR in order to place the ≥500 msec value in appropriate clinical context. However, values from individual tracings within triplicate measurements that are ≥500 msec will not be included in the categorical analysis

unless the average from the triplicate measurements is also ≥500 msec. Changes from baseline will be defined as the change between QTcF post-dose from the average of the last pre-dose triplicate values on the dosing day in each period.

6.1.5. Physical Examination and Neurological Examinations

Abnormal and clinically relevant changes in physical and neurological in AE tables by dose cohort and ambulatory status at baseline.

6.1.6. Cardiac MRI (or Echocardiogram, if necessary)

Cardiac MRI results including the LVEF, left arterial diameter, LV mass index, LV end-diastolic diameter, LV end-systolic diameter, fractional shortening, LV wall thickness, LV posterior wall thickness, tricuspid valvular regurgitation presence and pericardial effusion will be provided in a data listing. Changes from baseline in the following parameters will be summarized descriptively by dose cohort and ambulatory status at baseline:

- LVEF (%)
- LV Fractional Shortening (%)
- LV mass index
- Presence or absence of late gadolinium enhancement
- Observations from the cardiac MRI will be used for reporting. If cardiac MRI was not conducted at the scheduled visit (eg, due to young subject's inability to remain still during the scan), the results from the echocardiogram (if available for at least 3 participants with baseline assessments in the same dose cohort) will be summarized and listed separately.

6.1.7. Other Safety Data

Changes in weight and the C-SSRS will be summarized by dose cohort and ambulatory status at baseline.

6.2. Secondary Endpoint(s)

Quantification of the expression and distribution of mini-dystrophin detected by LC-MS and the immunofluorescence assay can provide evidence of expression of the transgene product. To limit possible variability by averaging or comparing results from different sections of a given muscle biopsy tissue, only sectioned tissue with results from each of the planned assays will be included in the summary.

The bootstrapping method (Efron 1979[2]) will be used to compute the 95% CIs of the parameters of interest for robust evaluation without underlying distributional assumptions.

6.2.1. Mini-dystrophin/Dystrophin Expression by LC-MS

Endpoints:

- Mini-dystrophin levels, expressed as fmol/mg of total protein, measured by LC-MS assay, LEMPSSLMLEVPTHR (LEMP) peptide
- Total dystrophin levels, expressed as fmol/mg of total protein, measured by LC-MS assay, LLQVAVEDR (LLQV) peptide
- Total dystrophin levels, expressed as percent normal, measured by LC-MS assay, LLQVAVEDR (LLQV) peptide

The analyses listed below will be performed for each of the 3 endpoints. The method for handling results below the LLOQ is described in Section 5.3.3. The analyses will be performed using the Pharmacodynamic Analysis Set. Presentations will include:

- Raw values, change from baseline and % change from baseline will be summarized descriptively by dose cohort, ambulatory status at baseline, and study visit.
- A boxplot (for cohort(s) with over 3 participants, data points display only for cohort with ≤3 participants) presenting the distribution of the change from baseline against study visit. A similar plot will also be generated for raw values and % change from baseline.
- An individual plot presenting raw levels against study visit, paged by dose cohort and ambulatory status at baseline. A similar plot will also be generated for the change from baseline.

6.2.2. Mini-dystrophin/Dystrophin Distribution by Immunofluorescence

Endpoints:

- Percent of dystrophin and mini-dystrophin positive muscle fibers derived from the individual fiber MSD data, measured by immunofluorescence assay using DYSB antibody (IF DYSB)
- Percent of mini-dystrophin positive muscle fiber derived from the individual fibers MSD data, measured by immunofluorescence assay using mini-dystrophin specific antibody (IF mini-dys)

The analyses listed below will be performed for each of the 2 endpoints. The analyses will be performed using the Pharmacodynamic Analysis Set. Presentations will include:

- Raw values, change from baseline and % change from baseline will be summarized descriptively by dose cohort, ambulatory status at baseline, and study visit.

- A boxplot (for cohort(s) with over 3 participants, data points display only for cohort with ≤3 participants) presenting the distribution of the change from baseline against study visit. A similar plot will also be generated for raw values and % change from baseline.

- An individual plot presenting raw levels against study visit, paged by dose cohort and ambulatory status at baseline. A similar plot will also be generated for the change from baseline.

Determination of % positive fibers from the immunofluorescence assay (DYSB and mini-dystrophin specific antibody):

The primary variables include the percent of positive fibers, the total number of fibers, and the percent of non-muscle tissue in biopsy. The determination of percent positive fibers is summarized as follows:

- Biopsy samples in stained slides will be sent to an independent vendor for processing. The digital image of each slide is generated.

- An ROA is outlined by pathologists in a platform.

- Each fiber membrane is scanned using a scanner. The whole biopsy quantitation of dystrophin mean myofiber MSD is determined for each fiber within the ROA. These data are called the individual fiber MSD data.

- The fiber MSD value for each fiber in the ROA is compared to a positive fiber threshold value to determine the number of fibers exceeding the threshold value within the ROA for each slide. The percent of positive fibers is computed as the number of positive fibers divided by the total number of fibers for each slide.

For each participant, the individual baseline fiber MSD method will be used to determine the threshold for positive fibers to better characterize the individual change from baseline. The distribution of the individual fiber MSD data will be examined and plotted. The 95th percentile of the baseline fiber MSD distribution for each slide obtained prior to dosing will be computed. The maximum of the 95th percentiles obtained from the baseline distribution(s) will be used as the threshold for positive fibers. This individual threshold of staining density will be established for each participant such that at least 95% of fibers have a staining density below the threshold for each slide analyzed at baseline.

For the newly developed mini-DYS/Laminin Image Analysis assay, a global threshold for calculating the percentage of mini-dystrophin positive fibers will be established based on all baseline samples from the 19 ambulatory participants. The quantification of the immunofluorescence intensity level (MSD) associated with the individual muscle fibers will be used to calculate the 99th percentile of the overall fiber MSD distribution. The

corresponding fiber MSD threshold value will be determined. Any muscle fiber with an MSD value above this calculated threshold value will be considered as a positive fiber.

At each scheduled visit of muscle biopsy, the average of the percent of positive fibers obtained in each slide will be computed. This percent of positive fibers will be summarized by dose cohort and ambulatory status at baseline. Key fiber MSD distribution statistics including the mean and the median will be summarized. In addition, non-parametric Wilcoxon test will be used to examine the change in fiber MSD distributions.

6.2.3. Long-term safety

To evaluate the long-term safety of the study treatment, analysis of the primary safety endpoints described in Section 6.1 will include all data through 5 years post-treatment. These include the incidence, severity, causal relationship of TEAEs and clinically significant safety findings.


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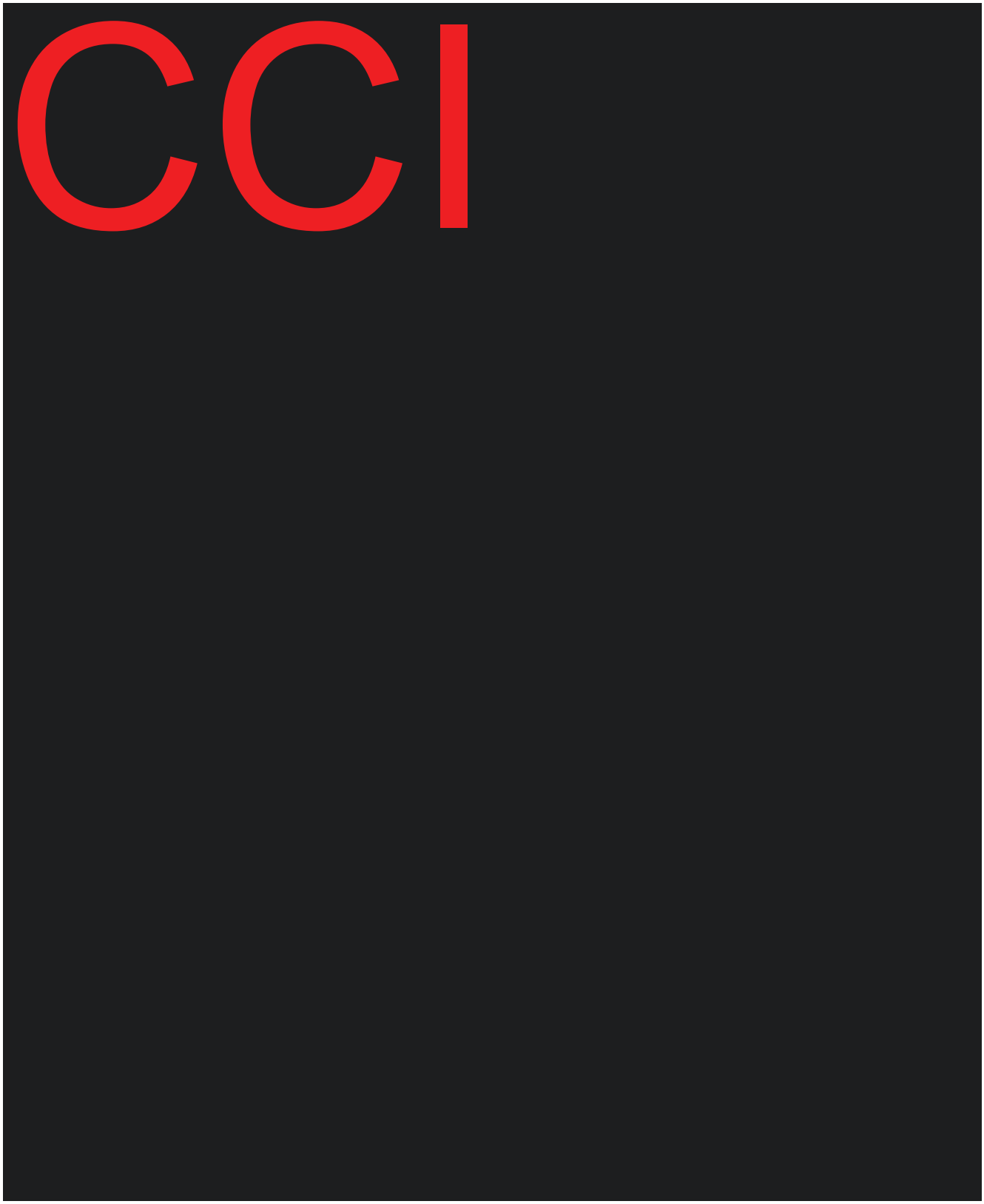
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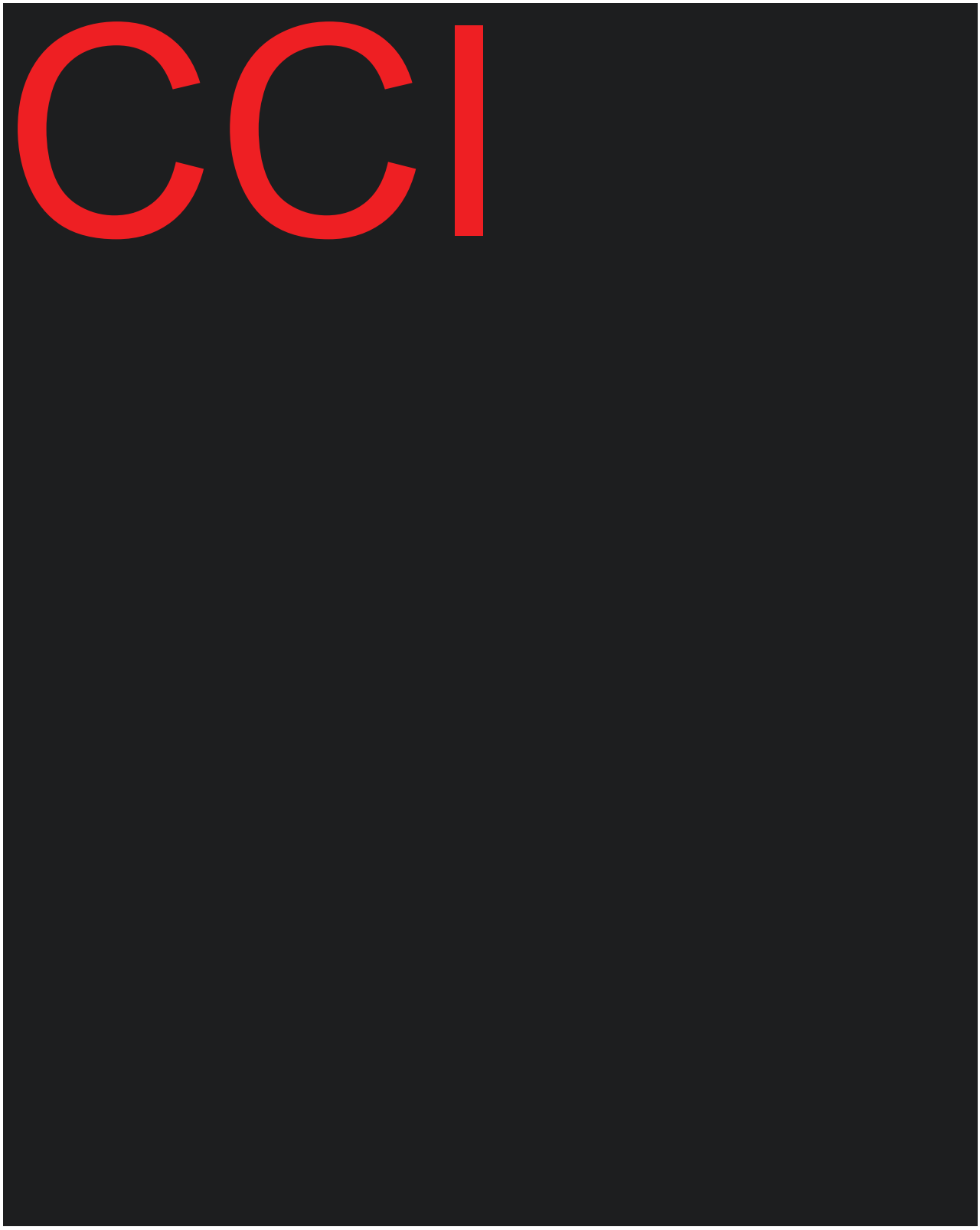
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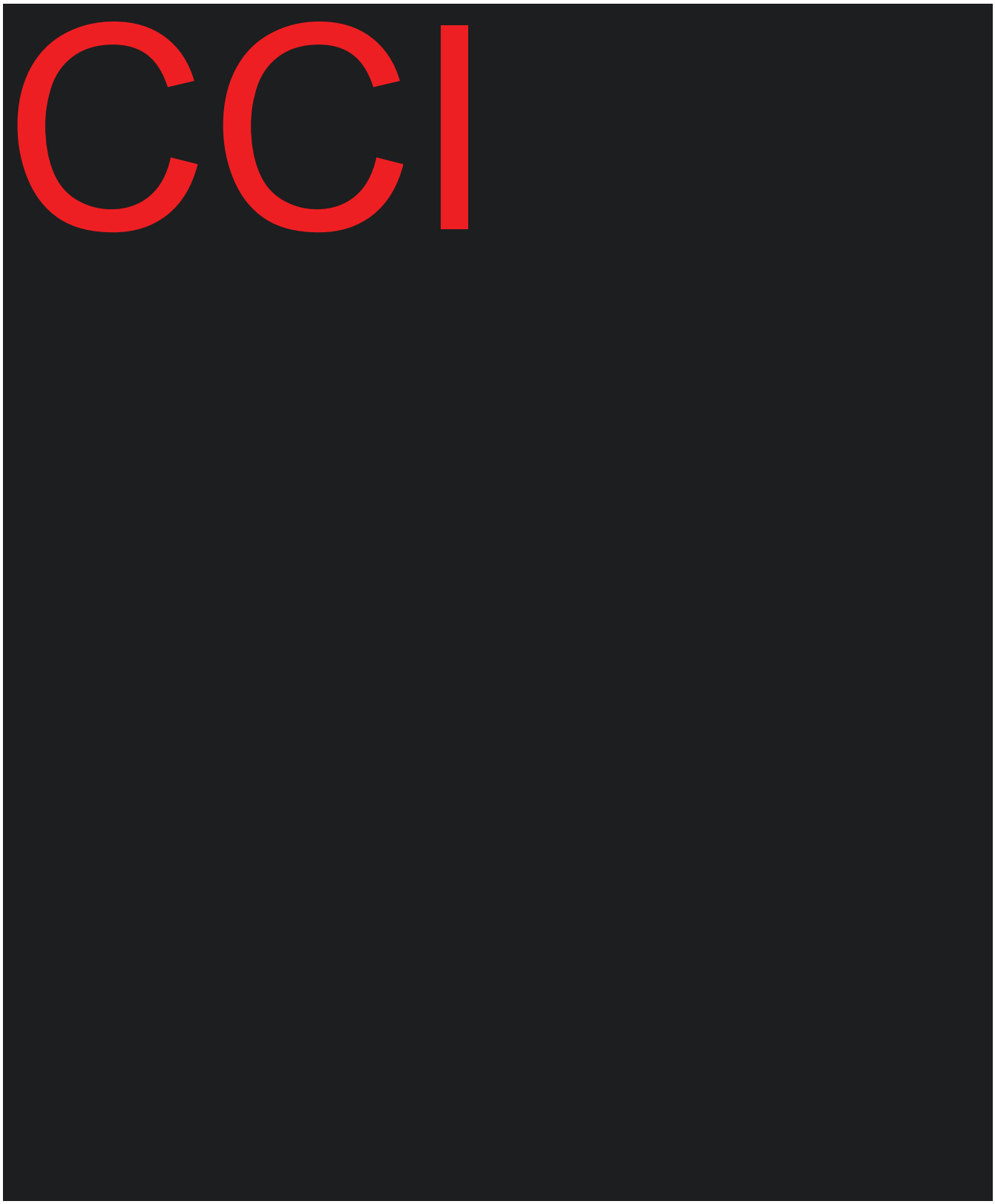
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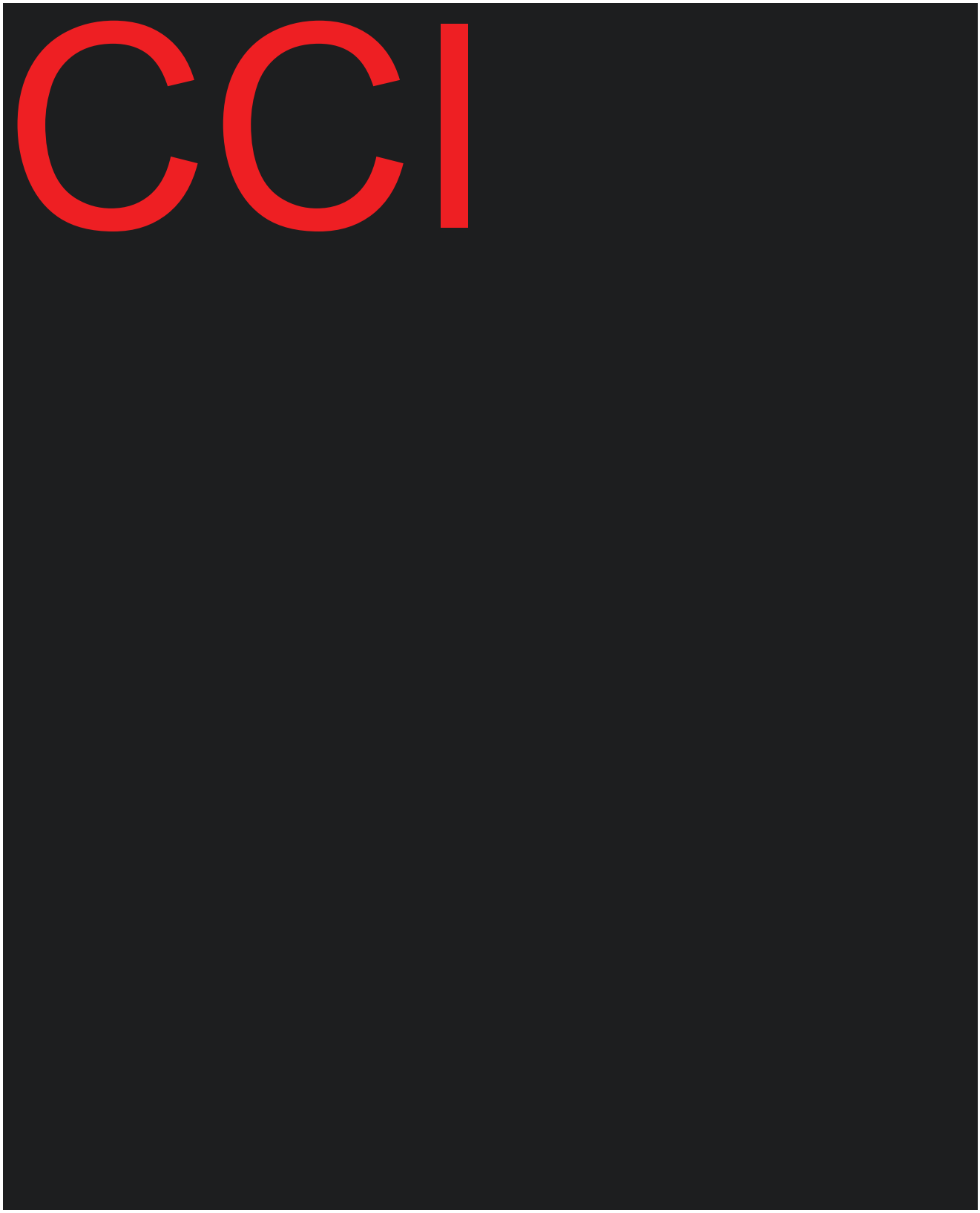
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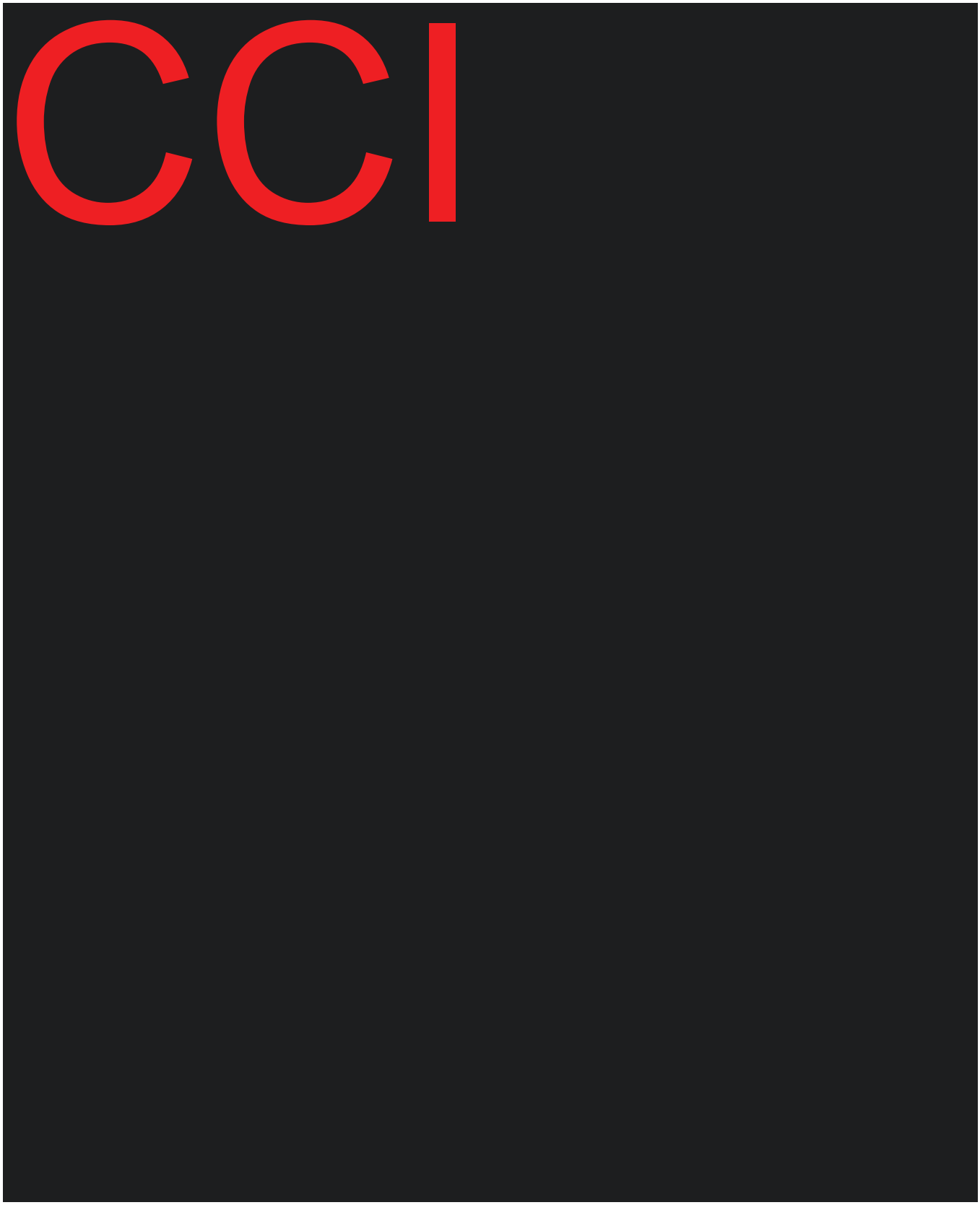
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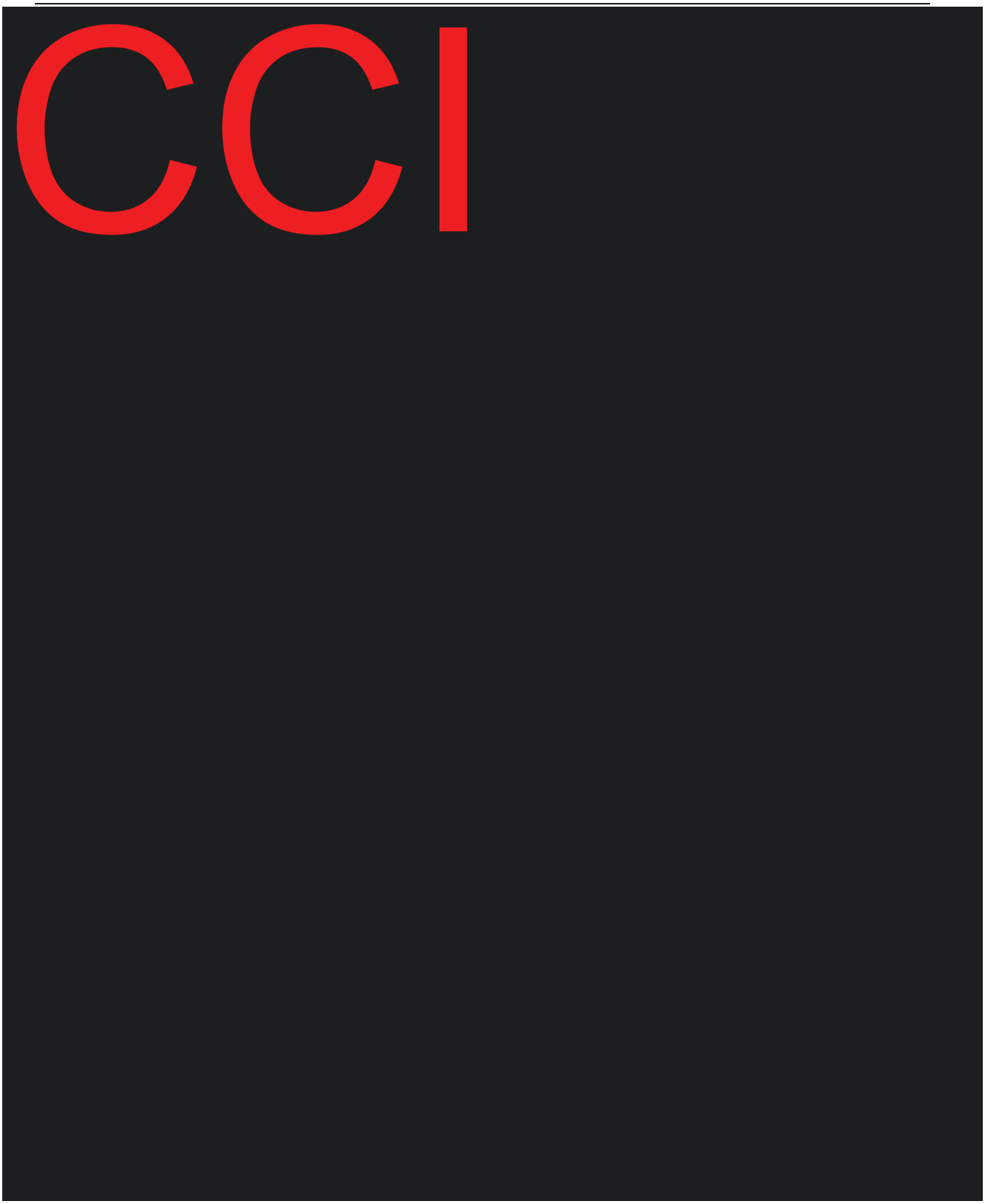
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6.4. Subset Analyses

6.4.2. Age Subgroups

Functional endpoints may be summarized separately for the age groups below.

- Age < 8 years
- Age ≥ 8 years

6.4.3. Participants with Specific Mutations

Listing of gene mutation data will be generated.

6.5. Baseline and Other Summaries and Analyses

6.5.1. Baseline Summaries

The following data will be summarized by dose cohort, ambulatory status at baseline, and overall.

1. Demographic characteristics will be summarized. Age categories to display are:
    - ≥4 to <8 years including subcategories of <5 years, ≥5 to <6 years, ≥6 to <7 years, and ≥7 to <8 years;

- ≥8 to ≤12 years including subcategories of ≥8 to <9 years, ≥9 to <10 years, ≥10 to <11 years, ≥11 to ≤12 years;
- >12 to <15 years, ≥15 to <18, ≥18 to <20 years, ≥20 to <25 years, and ≥25 years.

2. Age at dosing in years
3. Age at primary diagnosis in years
4. Race and Ethnicity
5. Physical measurements (including height, weight, body mass index)
6. Dystrophin mutation type
7. Background glucocorticoid regimen. Prednisolone and prednisone will be combined for this analysis. The number (%) of participants for each glucocorticoid being received at screening will be presented. Descriptive statistics will be presented for,
8. Daily dose (mg/kg) for each glucocorticoid the participant is receiving at screening. This will be calculated from the total daily dose and screening weight collected in the CRF;
9. Duration of glucocorticoid use (months) for glucocorticoid dose the participant is receiving at screening; and
10. Age at initiation of any glucocorticoid use.
11. General medical history
12. Prior medications

The following variables will be summarized using descriptive statistics,

- NSAA total score (ambulatory population only);
- Time to rise from floor (ambulatory population only);
- PUL 2.0 total score and each domain;
- %pFVC.

6.5.2. Study Conduct and Participant Disposition

Participant evaluation groups will show end of study participant disposition and will show which participants were analyzed for each analysis set. Frequency counts will be supplied for participant discontinuation(s) by dose cohort. Study Treatment Exposure

Study drug administration (fordadistrogene movaparvovec), as well as required concomitant and background medications, i.e. methylprednisolone infusion prior to fordadistrogene movaparvovec infusion, and glucocorticoid usage within and after 3 months post-fordadistrogene movaparvovec treatment will be provided in a listing.

Participant discontinuations will be listed and summarized by dose cohort. Data will be reported.

6.5.3. Concomitant Medications and Nondrug Treatments

The WHO-Drug coding dictionary will be used to classify concomitant medications.

All concomitant medication(s) as well as non-drug treatment(s) will be summarized as the number (%) of participants for each concomitant medication and non-drug treatment by dose cohort and ambulatory status at baseline.

6.6. Safety Summaries and Analyses

Primary safety analyses will be based on the data through 1-year post-treatment. Secondary safety analyses will be based on the long-term follow up data through 5-years post-treatment.

7. INTERIM ANALYSES

7.1. Introduction

No formal interim analysis will be conducted for this study. However, as this is an open label study, the sponsor and E-DMC may conduct unblinded reviews of the data during the course of the study for the purpose of safety assessment, facilitating dose escalation decisions, and/or to support clinical development. Mini-dystrophin expression and distribution will also be assessed at 2-month post-dose for the evaluation of dose progression and stopping rules. External trial data, when available and deemed appropriate, will be evaluated to contextualize the study finding and will be described in a supplementary SAP and reported separately from the CSR.

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7.2. Interim Analyses and Summaries

The E-DMC will be responsible for ongoing monitoring of the safety as well as benefit/risk profile of participants in the study. Reviews will include aggregate safety, targeted medical events of special interest, and serious AE data. The E-DMC may also complete ad hoc safety reviews of cohort data and/or individual participant data as requested by the study team as described in the E-DMC charter.

Following each data review, the E-DMC will provide a data-driven recommendation to the sponsor management to continue the study, modify the study and then continue (eg, terminate a dose level or de-escalate to a lower dose), or stop the study (eg, due to safety). The recommendations made by the E-DMC to alter the conduct of the study will be forwarded to Pfizer for final decision. Pfizer will forward such decisions, which may include summaries of aggregate analyses of endpoint events and of safety data that are not endpoints, to regulatory authorities, as appropriate. At any time, the E-DMC may indicate that the limit of safety and/or tolerability has been reached and that any of the dose levels will be removed from the study or adjusted.

8. APPENDICES

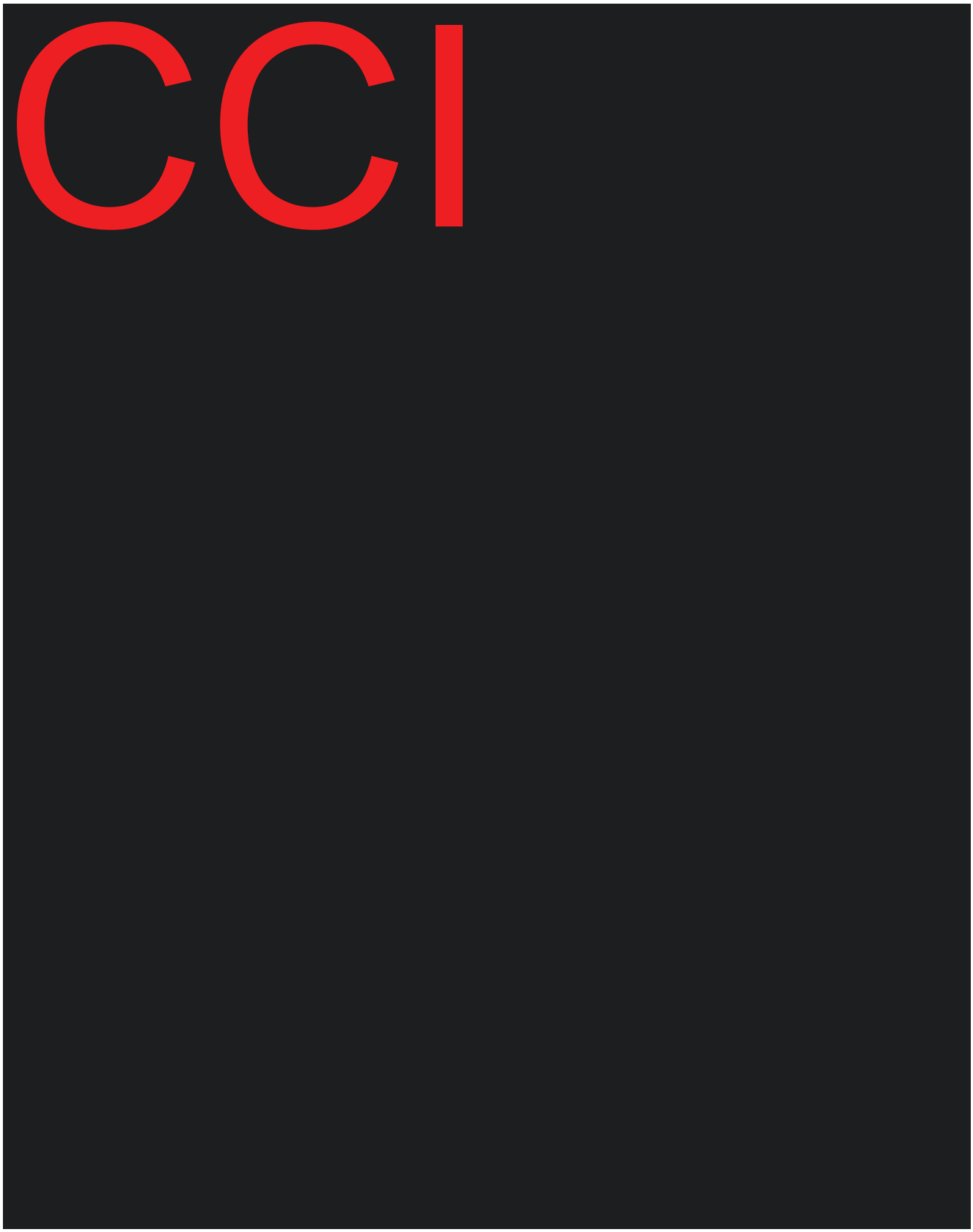
Appendix 1. Categorical Classes for ECG of Potential Clinical Concern

Categories for QTcF

	Borderline	Prolonged	
QTcF (ms)	450≤ max. <480	480≤ max.<500	max. ≥500
QTcF (ms) increase from baseline	30≤ max. <60	max. ≥60	

Categories for PR and QRS

PR (ms)	max. ≥300	
PR (ms) increase from baseline	Baseline >200 and max.≥25% increase	Baseline ≤200 and max. ≥50% increase
QRS (ms)	max. ≥140	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Appendix 3. SAP Amendment History

The SAP Amendment Summary of Changes Table for the current amendment is in Section 1. The SAP amendment summary of changes for past amendments can be found below.

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
1 16-Jan-2018	Original 20-Jun-2017	Not Applicable	Not Applicable
2 10-Feb-2020	Amendment 1 03-Dec-2018; Amendment 2 14-Feb-2019; Amendment 3 10-May-2019	Elaborated on the analyses of the biopsy data, the activity monitoring data, and the vector shedding data. Clarified languages for various analyses and added an external control section.	[redacted]
			Section 2.2: For this Study Design section, 1) Added the option of an intermediate dose level to which the second cohort may be de-escalated in the event of dose-limiting toxicity observed at the 3E+14 vg/kg dose level; 2) Revised the study schematic Figure 1; 3) For sample size, removed the wordings that "6 subjects in each dose cohort will participate", and added the option of 'up to 3 additional subjects" may be exposed to better understand the safety profile and/or any preliminary efficacy findings.
			Sections 3.2.1.1 and 6.2.1: Added details of the quantification of dystrophin expression using LC-MS assay and dystrophin distribution using Mean membrane Stain Density (MSD) measured by immunofluorescence.
			Section 3.4: Added the stratification factor of ambulatory status at baseline for all analyses, where applicable, and details of baseline variables to be summarized.
			Section 4: Added Section 4.6 Vector Shedding Analysis Set to this Analysis Sets section.
			Section 5.1: Added a description that hypothesis testing will be conducted when deemed appropriate to understand the potential treatment difference in secondary pharmacodynamic endpoints and to contextualize the study findings in [redacted] functional outcomes including NSAA total score with reference to external controls.

			Section 5.3: Added details regarding various imputation values for assay results below the limit of quantification.
			Section 6.2: Added the condition that only sectioned tissue with results from each of the three planned assays (LC-MS, Western blot, and immunofluorescence) will be included in the summary to limit possible variability by averaging or comparing results from different sections of a given muscle biopsy tissue.
			Section 6.2.2: Added the methods and analysis of the percent positive fiber derived from the distribution of individual fiber MSD data for dystrophin distribution quantified by the immunofluorescence assay, CCI
			Section 6.3.2.3: Added the methods to derive compliance measures and analysis details of various primary activity monitoring variables for the analysis of activity monitoring data. CCI
			Section 6.5: Added some details of the safety analyses and the handling of data obtained by triplicate ECGs.
			Section 7.1: Added this Section number and more details about the dose progression and stopping rules.
			Section 7.2: Added the description of using external control data to provide context and understanding of the study finding.
			Section 8.0: Added reference articles
			Appendix 2: Added this Appendix to describe the methods used to construct the external control group for the treatment study to contextualize the findings in C3391001 study
3 8-Mar-2021	Amendment 4 20-Feb-2020; Amendment 5 20-Jul-2020; Amendment 6 3-Mar-2021	Expanded study population to include non-ambulatory participants with Duchenne muscular dystrophy (DMD).	Amended the SAP using a template with specific sections 2.1.1 to 2.1.3 on estimands. Replace the term “subject” by “participant” if the term “participant” is interchangeable with the term “subject” in protocols or previous SAP versions. CCI

		Elaborated on the methods of analysis using external study data. Added sections to handle missing data or out of protocol-specified windows that may be due to COVID-19 pandemics. Added criteria to evaluate whether there is a difference in the compliance / usable activity monitoring data before and after COVID pandemics.	Section 2.2: 1) Revised the study schematic Figure 1; 2) Added description of the non-ambulatory population and elaborated on the dose levels. 3) Increased the total number of participants to be enrolled to approximately 30, per availability of drug product. 4) Increased the duration of length of the entire study from approximately 7 years to 8 years. 5) Elaborated on the sequence of enrollment and corresponding dose level. Specified the grouping of participants dosed by 3E14 vg/kg by ITR method and 2E14 vg/kg by Transgene method to the High Dose Group.
			CCI
			Revised languages to include non-ambulatory participants in all analyses presentation.
			Section 3.1.5: Added a description of echocardiogram and the analysis of LVEF.
			Added Section 3.3.1 to describe the viral vector shedding endpoints in detail. Defined viral clearance and elaborated on the analysis details in Section 6.3.5.
			Section 3.4: Added descriptions of the baseline variables to be summarized.
			Section 4: Presented the analysis populations in Table format. Added description of ambulatory, non-ambulatory, and historical control population.
			Section 5: Clarified the timing of the primary analysis and secondary analysis. Added description of hypothesis testing and the use of synthetic control in Section 5.1 Hypotheses and Decision Rules.
			Added Section 5.3.5. Missed visits or Assessments out of Protocol-specified Visit Windows to describe 1) the analysis method of data that may be affected by the COVID-19 pandemic; 2) extended visit windows to minimize missing data. Added the description "*Observations obtained during the COVID pandemics will be flagged.*" to Section 6.3.2.3 Activity monitoring data.
			Added Section 5.4 to elaborate on the methods to derive the synthetic control from the external studies.
			Section 6.2: Removed the condition that the results from all three planned assays (chromatography/mass spectrometry [LC-MS], Western blot, and immunofluorescence) are needed to be included in the summary because the expression and distribution of dystrophin will be primarily assessed by LC-MS and immunofluorescence.
			Moved the subsections from previously Section 6.5 Safety Summaries and Analyses to Section 6.1Primary Endpoints. Elaborate further on the primary safety analyses through 1 year post treatment. Added Section 6.2.3 Long-term safety to

			describe the safety analyses of primary endpoints through 5 year post-treatment. 
			Added Section 6.4 Subset Analysis per the new SAP format.
			Section 6.5.1: Added details of analysis variables to the Baseline Summaries.
			Section 8. Added reference articles.
			Appendix 2: Removed Study C3391005: *A single-site, prospective, natural history study to establish normative data of real-world activity measures using wearable monitors in ambulatory subjects boys with DMD* from the list of studies used to construct the synthetic control group. This study has been terminated early due to the impact of COVID-19 pandemic on participant enrollment, compliance with in-clinic visits, and safety consideration during travel to the study site. Two participants were enrolled at the time of study termination. Added Study C3391004: *A Natural History Study In Chinese Male Patients With Duchenne Muscular Dystrophy*. This is a multicenter, prospective study of approximately 330 ambulatory and non-ambulatory participants. Elaborated on the eligibility criteria for the synthetic control.
			Added Appendix 3. Definition and use of visit windows in reporting and Appendix 4. List of Abbreviations.
4 25-Oct-2021	Amendment 7 3-Jun-2021; Amendment 8 2-Sep-2021	Expanded study population to include Sirolimus cohort.	Section 2.1: Added Section 2.1.4 “Estimand(s) for Sirolimus Evaluation” to describe the objectives, endpoints and attributes associated with the estimands.
			Section 2.2: 1) Revised the study schematic Figure 1; 2) Added the Sirolimus Cohort to the sequence of enrollment.

		Added analyses to evaluate the effect of Sirolimus. Clarified SAP content.	3) Increased the total number of participants to be enrolled to approximately 35, of which up to 25 participants are expected to be ambulatory upon study entry.
			Section 3.1.1: Added a description of treatment-emergent adverse events for the evaluation of safety in the Sirolimus cohort.
			Section 4: Added a description of Sirolimus cohort and non-sirolimus cohort.
			Section 5.1: Clarified the timing of the primary analysis and interim analysis when study participants completed 1 year of follow-up.
			Section 6.1.1: Added the analysis of the incidence and severity of TEAEs (all causalities and treatment-related) through 30 days post the last dose of sirolimus administration for those participants who received sirolimus (Sirolimus cohort) versus those who did not (non-sirolimus cohort).
			Section 6.1.2: 1) Added the analysis of the incidence and magnitude of abnormal laboratory findings through 30 days post the last dose of sirolimus administration by Sirolimus cohort status. 2) Described the additional analysis of C4 and platelet levels to assess the effect of sirolimus. 3) Clarified the criteria used to identify potential liver injury cases from the eDISH plot.
			Section 6.3.1: 1) Elaborated on the definition of the ELISpot overall positive or negative immune response. 2) Added the description that the observed NAb antibody titers will be summarized and graphically presented by dose cohort, ambulatory status, and sirolimus cohort status.
			Section 7.3: 1) Changed “first” cohort to “low dose” cohort and “second” cohort to “high dose” cohort. 2) Changed GLDH >2.5 × ULN to “Any set of laboratory results meeting Hy’s Law” for the criteria to halt further enrollment, and clarified that the E-DMC will review data for the participants with the clinical events that prompted the review instead of all available data.
			Appendix 3: Clarified the extended time window for various assessments.
			Appendix 4: Added TBili (total bilirubin) to the glossary.
5 11-May-2022	Amendment 9 7-Mar-2022	The study will no longer enroll subjects. Clarified analyses of non-	Section 2.2: 1) Revised the total number of participants to include up to 22 DMD participants (19 ambulatory and 3 non-ambulatory participants). 2) Revised the study schematic Figure 1. 3) Revised the number of participants in the sirolimus cohort.
			Section 3.2.1.1 and Section 3.2.1.2:

		ambulatory subjects with n ≤ 3. Added the description of a newly developed mini-DYS/Laminin assay to quantify the mini-dystrophin distribution. Revised the extended time window at Day 360. Clarified SAP content.	Added the description of using a newly developed mini-DYS/Laminin Image Analysis assay to evaluate the mini-dystrophin distribution and the corresponding baseline definition.
			Section 4: Added "(or External Control)" to the Synthetic Control description.
			Section 5: Added "For the analyses of a subgroup with <3 participants, individual patient profile descriptive analyses will be conducted instead of summary statistics across the subgroup.
			Section 5.1: Clarified the timing of the primary analysis and interim analysis when study participants completed 1 year of follow-up.
			Section 5.2: Added the correlation and/or regression analysis between various endpoints of interest will be conducted if deemed appropriate. Spearman's rank correlation will be used to assess monotonic relationships and Pearson's correlation will be used to assess linear relationships. Regression models may be used with additional covariates if deemed appropriate. CCI [redacted]
			Section 6.1.2: Added "if there are at least 3 participants in each cohort" to specify when the summary analyses by sirolimus cohort (sirolimus, non-sirolimus) will be conducted. Removed "The two-sample non-parametric Wilcoxon test will be used to evaluate the difference in the percent change from baseline to nadir through Day 30 visit post PF-06939926 infusion between the sirolimus cohort and the non-sirolimus cohort."
			Section 6.2.2: Added "For the newly developed mini-DYS/Laminin Image Analysis assay, a global threshold for calculating the percentage of mini-dystrophin positive fibers will be established based on all baseline samples from the 19 ambulatory participants. The quantification of the immunofluorescence intensity level (Mean Stain Density, MSD) associated with the individual muscle fibers will be used to calculate the 99th percentile of the overall MSD distribution. The corresponding MSD threshold value will be determined. Any muscle fiber with an MSD value above this calculated threshold value will be considered as a positive fiber."
			Section 6.3.1: Added "(if there are at least 3 participants in each group)" to specify when the observed NAb antibody titers will be

			summarized and graphically presented by dose cohort, ambulatory status, and sirolimus cohort status.
			Section 6.3.2: Added the analysis of the ambulatory health states and the corresponding definition based on the NSAA assessments
			Section 6.3.2.1: Elaborated on the analysis of the number of functional skills gained, improved, or maintained based on the NSAA assessment.
			Section 6.3.2.3: Relationship between selected activity monitoring outcome measures (eg. the 95th percentile of the cadence) and the in-clinic functional assessments (eg. NSAA total score) will be explored descriptively or via regression modeling approaches.
			Section 6.3.3: Added that the relationship between selected MRI parameters of interest (eg. muscle volume, mean fat fraction) and the in-clinic functional assessments (eg. NSAA total score) will be explored descriptively or via regression modeling approaches.
			Section 7: Clarified the timing of the primary safety analyses.
			Appendix 3: Extended the time window at Day 360 from +30 days to +45 Days.
			Appendix 4: SAP amendment History Summary of changes for past SAP amendments.

Appendix 4. List of Abbreviations

Abbreviation	Term
%pFVC	percent predicted forced vital capacity
4SC	four stair climb
6MWD	six minute walk distance
AAV9	adeno-associated virus serotype 9
ADA	anti-drug antibodies
AE	adverse event
ALT	alanine aminotransferase
ALP	alkaline phosphatase
AST	aspartate aminotransferase
CI	confidence interval
CK	creatine kinase
cTn-I	cardiac troponin I
COVID-19	Coronavirus Disease 2019
CRF	case report form
CSR	Clinical study report
C-SSRS	Columbia Suicide Severity Rating Scale
DMD	Duchenne muscular dystrophy
eDISH	evaluation of Drug-Induced Serious Hepatotoxicity
ECG	electrocardiogram
E-DMC	external data monitoring committee
ELISpot	enzyme-linked immune absorbent spot
EQ-5D	EuroQol 5 Dimensions
[redacted]	[redacted]
EQ VAS	EuorQol visual analogue scale
FAS	full analysis set
FDA	Food and Drug Administration (United States)
FEV_1	forced expiratory volume in one second
FEV_6	forced expiratory volume in six seconds
FIH	first-in-human
FIP	first-in-patient
FVC	forced vital capacity
GGT	gamma-glutamyl transferase
GLDH	glutamate dehydrogenase
hCK	hybrid creatine kinase
ICH	International Council for Harmonisation
IF	immunofluorescence
ITR	inverted terminal repeats
IV	intravenous

Abbreviation	Term
LC-MS	liquid chromatography-mass spectrometry
LEMP	LEMPSSLMLEVPTHR
LLN	lower limit of normal
LLOQ	lower limit of quantitation
LLQV	LLQVAVEDR
LS	least squares
LV	left ventricular
LVEF	left ventricular ejection fraction
MedDRA	Medical Dictionary for Regulatory Activities
MFI	mean fluorescence intensity
MNAse	micrococcal nuclease
MRI	magnetic resonance imaging
MSD	mean stain density
NAb	neutralizing antibodies
NSAA	North Star Ambulatory Assessment
PFT	pulmonary function tests
PODCI	Pediatric Outcomes Data Collection Instrument
PT	(MedDRA) Preferred Term
PUL	Performance of Upper Limb
Q1	first quartile
Q3	third quartile
qPCR	quantitative polymerase chain reaction
QTc	corrected QT
QTcF	QTc corrected using Fridericia's formula
ROA	region of analysis
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SOC	system organ class
TBili	total bilirubin
TEAE	treatment-emergent adverse event
TG	transgene
ULN	upper limit of normal
ULOQ	upper limit of quantitation
CCI [redacted]	
US	United States
vg	vector genomes
WHO	World Health Organization
CCI [redacted]	

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