Protocol C3391007

A LOW-INTERVENTIONAL STUDY OF AAV9 NEUTRALIZING ANTIBODY SEROCONVERSION IN HOUSEHOLD CONTACTS OF PARTICIPANTS WITHIN FORDADISTROGENE MOVAPARVOVEC CLINICAL TRIALS

Statistical Analysis Plan
(SAP)

Version: **3**

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1. VERSION HISTORY

This Statistical Analysis Plan (SAP) V3 for study C3391007 is based on the protocol amendment #1 dated 05 Apr 2022.

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
V3/ 17 May 2024	Protocol amendment #1	To clarify that height was not collected according to protocol and age category summary is not needed due to small sample size	Section 3.4 Removed age category groups in demographic characteristics Removed height and BMI measurement in baseline summary
		To update statistical analysis method due to small number of enrolled patients	Section 5.2.2 Removed model based analysis for confidence interval for proportion Sections 6.1, 6.2, 6.3 Removed GEE analysis and 95%CI from the analysis and summary Section 6.4 Removed subgroup analysis
		To clarify the summary results needed for interim analysis	Section 7 Added “In order to minimize possibility of unblinding internal study team, the external vendor will provide only the percent of participants whose family members received fordadistrogene movaparvovec and converted.”

2. INTRODUCTION

This statistical analysis plan (SAP) provides the detailed methodology for summary and statistical analyses of the data collected in Study C3391007. 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.

This SAP covers the analyses to be performed for the primary analysis that is to occur when all participants have completed visits through 4 months (or withdrawn from the study prior to 4 months). The C3391007 Data Access Plan describes the reporting strategy that appropriately maintains the blinded treatment assignment in Studies C3391003 and C3391002.

Statistical analyses will be performed using cleaned case report form (CRF) data as well as non-CRF data (i.e., blood samples data and banked biospecimens).

2.1. Study Objectives and Endpoints

Primary Objective:	**Primary Endpoint:**
To quantify the proportion of participants (previously seronegative for neutralizing antibody [NAb] to adeno associated virus serotype 9 [AAV9]) who develop NAb to AAV9 (i.e., seroconversion)	Development of NAb to AAV9 (i.e., NAb seroconversion) as defined by an increase of ≥6-fold above Baseline titer in participants with a detectable, but negative test (i.e., titer ≥1 and <4) for NAb to AAV9 at Baseline. If there is an undetectable titer at Baseline (i.e., titer <1), then development of NAb to AAV9 as defined by a NAb to AAV9 titer.
Secondary Objective:	**Secondary Endpoint:**
To quantify the proportion of participants (previously seronegative for anti-drug antibody [ADA] to AAV9) who develop ADA to AAV9.	Development of ADA to AAV9 (i.e., ADA seroconversion) defined by a titer ≥300 in participants with a negative test (i.e., titer <50) for ADA to AAV9 at Baseline.
Exploratory Objectives:	**Exploratory Endpoints:**
To quantify the proportion of participants (previously seropositive for NAb to AAV9) who have an increase in the NAb to AAV9 titer.	NAb to AAV9 titer ≥6-fold higher than Baseline titer in participants who are seropositive (i.e., titer ≥4) for NAb to AAV9 at Baseline.
To quantify the proportion of participants (previously seropositive for ADA to AAV9) who have an increase in the ADA to AAV9 titer.	ADA to AAV9 titer ≥6-fold higher than Baseline titer in participants who are seropositive (i.e., titer ≥50) for ADA to AAV9 at Baseline.

To quantify the proportion of participants who seroconvert and have detectable fordadistrogene movaparvovec vector deoxyribonucleic acid (DNA) in blood.	Detectable fordadistrogene movaparvovec vector DNA in participants who seroconvert via either ADAs or NAbs.

Note that the NAb analytical report delivers a titer value (based on interpolation of NAb activity at several dilutions), as well as a clinical assessment of "positive" or "negative" based on the NAb activity at a specific 1:4 dilution. Because of the potential for discrepancy between the quantitative result and the clinical result, the clinical result will be disregarded in this study and all analyses will be derived based on the titer value from the report.

2.2. Study Design

C3391007 is single center, low-interventional study to include approximately 50 to 250 participants and is designed to estimate the likelihood of NAb seroconversion to AAV9 because of exposure to shed viral vector material released by a Duchenne muscular dystrophy (DMD) participant treated with fordadistrogene movaparvovec in clinical studies. This study will include participants who live or work in the same household as a participant in one of the fordadistrogene movaparvovec interventional studies, which may include study C3391003, C3391002, C3391008, or C3391001. Up to 5 participants from the same household may be enrolled.

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

Note that the NAb analytical report delivers a titer value (based on interpolation of NAb activity at several dilutions), as well as a clinical assessment of "positive" or "negative" based on the NAb activity at a specific 1:4 dilution. Because of the potential for discrepancy between the quantitative result and the clinical result, the clinical result will be disregarded in this study and all analyses will be derived based on the titer value from the report.

3.1. Primary Endpoint

- Development of NAb to AAV9 (i.e., NAb seroconversion) defined as either of the following:
    - an increase of ≥ 6-fold above Baseline titer in participants with a detectable, but negative test (i.e., titer ≥ 1 and < 4) for NAb to AAV9 at Baseline or
    - a NAb to AAV9 titer ≥6 in participants with an undetectable titer at Baseline (i.e., titer <1).

The response of developing NAb seroconversion will be derived as Positive or Negative.

3.2. Secondary Endpoint

- Development of ADA to AAV9 (i.e., ADA seroconversion) defined by a titer ≥300 in participants with a negative test (i.e., titer < 50) for ADA to AAV9 at Baseline.

The response of developing ADA seroconversion will be derived as Positive or Negative.

3.3. Exploratory Endpoints

- Boosted NAb to AAV9 defined by NAb to AAV9 titer ≥6-fold higher than Baseline titer in participants who are seropositive (i.e., titer ≥4) for NAb to AAV9 at Baseline.
    - Note that the upper limit of quantification of AAV9 NAb titer is 32,000, and if the baseline titer is >5,333, a 6-fold increase of titer cannot be determined. Therefore, NAb seropositive subjects with baseline NAb titer >5,333 will be removed from this particular exploratory analysis.
- Boosted ADA to AAV9 defined by ADA to AAV9 titer ≥6-fold higher than Baseline titer in participants who are seropositive (i.e., titer ≥50) for ADA to AAV9 at Baseline.
- Detectable fordadistrogene movaparvovec vector DNA in blood defined as a detectable fordadistrogene movaparvovec vector DNA in participants who seroconvert via either ADAs or NAbs.

The responses of the exploratory endpoints above will be derived as Positive or Negative.

3.4. Baseline Variables

Baseline will be defined as the last value collected prior to the start of study treatment (Day 1) of the interventional study participant.

- Demographic characteristics
    - Gender: Male, Female
    - Race: White, Black or African American, Asian, American Indian or Alaska Native, Native Hawaiian or Other Pacific Islander, Not Reported
    - Ethnic origin: Hispanic or Latino or of Spanish Origin, Not Hispanic or Latino or of Spanish Origin, Not Reported
    - Age (years)
    - Relationship to the interventional study participant
- Physical measurements
    - Weight (kg)
- Medical history will be coded using the most current available version of Medical Dictionary for Regulatory Activities (MedDRA) and will be summarized from the 'Medical History' CRF page.

- Prior Medications at baseline are collected under the 'Concomitant treatments' CRF pages (Visit 1). The World Health Organization (WHO)-Drug coding dictionary will be used to classify concomitant medications.

3.5. Study Conduct and Participant Disposition

3.5.1. Extent of Contact with the Participant in the Interventional Study

- The average number of hours per week that the participant is in the same room as the interventional study participant at visit 1 (screening and baseline visit) is derived as described in Appendix 2.2.

- Total number of days of contact in the same room as the interventional study participant during the first two weeks after administration of study treatment.

3.6. Safety Endpoints

3.6.1. Adverse Events

MedDRA will be used to classify all adverse events (AEs) with respect to system organ class (SOC) and preferred term (PT). Any AE that occurs from the time the participant consents to study participation will be summarized regardless of when the interventional study participant is dosed with study treatment.

3.6.2. Laboratory Data

No safety laboratory tests are performed in this study and analyses for banked biospecimens are out of scope for this SAP.

3.6.3. Concomitant Medications

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

4. ANALYSIS SETS (POPULATIONS FOR ANALYSIS)

Data for all participants will be assessed to determine if participants meet the criteria for inclusion in each analysis population prior to releasing the database and classifications will be documented per standard operating procedures.

Only participants who signed informed consent will be included in the analysis sets below.

Population	Description
Immunogenicity	All participants who sign the informed consent document: 1) who are household members of interventional study participants (including siblings) who were assigned to fordadistrogene movaparvovec or placebo and received a single dose of study treatment and 2) who have at least one day of contact with the interventional study participant during the first 2 weeks.

5. GENERAL METHODOLOGY AND CONVENTIONS

5.1. Hypotheses and Decision Rules

There is no formal hypothesis testing planned for this study.

5.2. General Methods

For analyses which consider an overall assessment of rates across timepoints, a participant will be considered to have a positive result, if there is at least one positive result after Day 1; similar a participant will be considered to have a negative result if all results after Day 1 are negative.

5.2.1. Presentation of Continuous and Qualitative Variables

Continuous variables will be summarized using descriptive statistics i.e., number of non-missing values N, mean, median, standard deviation (SD), minimum, maximum and first and third quartile (Q1 and Q3).

Qualitative variables will be summarized by frequency counts and percentages.

In case the analysis refers only to certain visits, percentages will be based on the number of participants with non-missing results at that visit, unless otherwise specified.

5.2.2. Analyses for Proportions (%)

The proportion ($\hat{p}_t$) will be provided for interventional study treatment and each visit.

$\hat{p}_t = \frac{x_t}{n_t}$, where n_t is the number of household contacts in each interventional study treatment and visit and x_t is the number of seroconversion (positive) of household contacts in each interventional study treatment and visit.

5.3. Methods to Manage Missing Data

Unless otherwise specified, all data will be evaluated as observed, and no imputation method for missing values will be used.

Missing statistics, e.g. when they cannot be calculated, should be presented as 'Not applicable' (NA). For example, if N=1, the measure of variability (SD) cannot be computed and should be presented as 'NA'.

6. ANALYSES AND SUMMARIES

6.1. Primary Endpoint

6.1.1. Development of NAb to AAV9

- Analysis Set: Immunogenicity (Section 4)
- Analysis methodology: Descriptive statistics (Section 5.2.1) by visit and overall
- Missing data: Missing data will not be imputed (Section 5.3)

- Proportions will be provided separately for visits 3 and 4, and overall at any time during the study by the study treatment (fordadistrogene movaparvovec fordadistrogene movaparvovec or placebo) of the interventional study participant. For overall, the response of the participant will be considered as positive if any values in visits 3 and 4 are positive; otherwise the response will be considered as negative.

6.2. Secondary Endpoint

6.2.1. Development of ADA to AAV9

- Analysis Set: Immunogenicity (Section 4)
- Analysis methodology: Descriptive statistics (Section 5.2.1) by visit and overall
- Missing data: Missing data will not be imputed (Section 5.3)
- Proportions will be provided separately for visits 3 and 4, and overall at any time during the study by the study treatment (fordadistrogene movaparvovec or placebo) of the interventional study participant. For overall, the response of the participant will be considered as positive if any values in visits 3 and 4 are positive; otherwise the response will be considered as negative.

6.3. Exploratory Endpoints

- Boosted NAb to AAV9.
- Boosted ADA to AAV9.
- Detectable fordadistrogene movaparvovec vector DNA.

For each of the endpoints listed above:

- Analysis Set: Immunogenicity (Section 4)
- Analysis methodology: Descriptive statistics (Section 5.2.1)by visit and overall
- Missing data: Missing data will not be imputed (Section 5.3)
- Proportions will be provided separately for visits 3 and 4, and overall. at any time during the study by the study treatment (fordadistrogene movaparvovec or placebo) of the interventional study participant. For overall, the response of the participant will be considered as positive if any values in visits 3 and 4 are positive; otherwise the response will be considered as negative.

6.4. Subgroup Analyses

Not applicable.

6.5. Baseline and Other Summaries and Analyses

6.5.1. Baseline Summaries

6.5.1.1. Demographic Characteristics

Demographic characteristics and physical measurements will be summarized for all participants and by the interventional study participant's treatment group (fordadistrogene movaparvovec or placebo).

6.5.1.2. Medical History

Medical history will be summarized as the numbers and percentage of participants by MedDRA preferred term (PT) as event category and MedDRA primary system organ class (SOC) as summary category for all participants and by the interventional study participant's treatment group (fordadistrogene movaparvovec or placebo). Each participant will be counted only once within each PT or SOC.

6.5.1.3. Prior Medications

Prior medications will be summarized as the number and percentage of participants by WHO dictionary term for all participants and by the interventional study participant's treatment group (fordadistrogene movaparvovec or placebo).

6.5.2. Study Conduct and Participant Disposition

Participant disposition (e.g., discontinuation from study, reason for discontinuation, completed study, and ongoing) will be summarized by the interventional study participant's treatment group (fordadistrogene movaparvovec or placebo).

6.5.2.1. Extent of Participant Contact

The derived average number of hours per week that the participant is in the same room as the interventional study participants at visit 1 (screening and baseline visit) will be summarized as described in Section 5.2.1.

Number of days of contact in the same room as the interventional study participant during the first two weeks after administration of study treatment will be summarized at visit 2 by the interventional study participant's treatment group (fordadistrogene movaparvovec or placebo) as described in Section 5.2.1.

6.5.3. Study Treatment Exposure

Participants in this study do not receive study treatment and thus, there is no formal exposure analysis planned.

6.5.4. Concomitant Medications

The number and percent of participants who took each concomitant medication will be provided by the interventional study participant's treatment group (fordadistrogene movaparvovec or placebo).

6.6. Safety Summaries and Analyses

6.6.1. Adverse Events

The overall summary of AEs will include the frequency (number and percentage) and severity of AEs by SOC and PT by the interventional study participant's treatment group (fordadistrogene movaparvovec or placebo).

Serious AEs will be listed.

6.6.2. Laboratory Data

Not applicable.

7. INTERIM ANALYSES

To enable potentially informing participants and investigators of the estimated seroconversion rate as soon as possible, an independent, external team of statistician(s) and programmer(s) will be established to estimate the seroconversion rates. Only summary seroconversion rates will be communicated. In order to minimize possibility of unblinding internal study team, the external vendor will provide only the percent of participants whose family members received fordadistrogene movaparvovec and converted.

8. REFERENCES

1. Liang, K.-Y., and Zeger, S. L. (1986). "Longitudinal Data Analysis Using Generalized Linear Models." Biometrika 73:13–22.

9. APPENDICES

Appendix 1. Summary of Efficacy Analyses

Not applicable.

Appendix 2. Data Derivation Details

Appendix 2.1. Definition and Use of Visit Windows in Reporting

The below analysis windows will be used.

Safety Parameter	Visit	Target day[1]	Protocol window lower limit	Protocol window upper limit	Analysis window
-Informed consent/assent -Review of incl/excl criteria -Medical history -Demographic information -Weight measurement -Banked biospecimens	1	1			
Extent of contact with the participant in the interventional study	1	1			
	2	Day +14	-0 days	+2 days	
-Concomitant treatments -Adverse event monitoring -Blood Samples for NAb and ADA to AAV9[2] -fordadistrogene movaparvovec vector DNA[2]	1	1			
	2	Day +14	-0 days	+2 days	
	3	Day +28	- 8 days	+ 8 days	15 - 42
	4	Day +56	- 10 days	+ 10 days	≥43

[1] Day relative to start of study treatment (Day 1) of the interventional study participant who is in the same household as the study participant.
[2] If multiple values are in a window the response of the participant will be considered as positive if any values in this window are positive; otherwise the response will be considered as negative.

The following conversion factors will be used to convert days into weeks, months or years: 1 week = 7 days, 1 month = 30.4375 days, 1 year = 365.25 days.

Appendix 2.2. Endpoint Derivations

For the extent of contact with the participant in the interventional study, the CRF uses a drop-down selection at baseline (see the table below) that will be converted into a continuous variable as follows:

Table: Numeric transformation for derived average hours of contact per week

Average number of hours of contact per week as collected in CRF at visit 1	Derived average number of hours of contact per week

10 - 12	11
13 - 16	14.5
17 - 20	18.5
21 - 24	22.5
25 - 30	27.5
31 - 36	33.5
37 - 42	39.5
43 - 48	45.5
49 - 60	54.5
61 - 72	66.5
73 - 84	78.5
85 - 96	90.5
97 - 120	108.5
121 - 144	132.5
145 - 168	156.5

Appendix 2.3. Definition of Protocol Deviations that Relate to Statistical Analyses/Populations

Not applicable.

Appendix 3. Data Set Descriptions

None.

Appendix 4. Statistical Methodology Details

None.

Appendix 5. SAP Amendment History

The summary of changes for the current SAP amendment are described in Section **Error! Reference source not found.**. The summary of changes for past amendments can be found below.

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
19 May 2020			*Original SAP*
V2/20Apr2022	protocol amendment #1	Updates following protocol amendment	Global updates: • Changed 'C3391003' to the 'interventional study' (which may include study C3391003, C3391002, C3391008, or C3391001)

			• Changed PF-06939926 to fordadistrogene movaparvovec
			Protocol title Modified ‘substudy’ to ‘study’
			Section 2.1 Removed ‘and have a household contact being treated with PF-06939926 or placebo in Study C3391003’ from study objectives
		SAP content clarification	Section 4 and 6 Removed ‘Enrolled population’ Modified ‘Safety’ population to ‘Immunogenicity’
			Section 5.2 Modified wordings for overall assessment
			Section 5.2.2.1 Added the statistical method for calculating observed proportions (95% CIs) and the difference in observed proportions (95% CI)
			Section 5.2.2.2 Clarified the approach for handling GEE model convergence issues
			Section 6 Added descriptive statistics in addition to GEE model estimates for primary, secondary and exploratory endpoints
			Section 6.1 and 6.2 Added descriptive statistics in the analysis methodology for the primary and secondary endpoints
			Section 6.5.4 Removed ‘according to Pfizer data standards’
			Section 7 Added texts to clarify the interim analysis
			Appendix 2.2 Modified the column names of the table

Appendix 6. List of Abbreviations

Abbreviation	Term
AAV9	adeno associated virus serotype 9
ADA	anti-drug antibody
AE	adverse event
BMI	body mass index
CI	confidence interval
CRF	case report form
DMD	Duchenne muscular dystrophy
DNA	deoxyribonucleic acid
GEE	generalized estimating equation
MedDRA	medical dictionary for regulatory activities
NA	not applicable
NAb	neutralizing antibody
NE	non-estimable
PT	preferred term
Q1	first quartile
Q3	third quartile
SAP	statistical analysis plan
SD	standard deviation
SE	standard error
SOC	system organ class
WHO	World Health Organization