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

Investigational Product Number:	PF-06939926
Investigational Product Name:	Not Applicable (NA)
United States (US) Investigational New Drug (IND) Number:	CCI
European Clinical Trials Database (EudraCT) Number:	NA
Protocol Number:	C3391001
Phase:	1b

Document History

Document	Version Date	Summary of Changes and Rationale
Amendment 9	07 March 2022	In Sections 1.4, 3.1, 3.2, and 9.1, changed the total N number to 22 which is the number of subjects who were dosed. • Rationale: The C3391001 study will no longer enroll subjects. In Section 7.2.12 (cTnI), added new parameters for the troponin level for the triggers to implement additional safety monitoring. • Rationale: Given that the central laboratory is using a new cardiac troponin assay (Beckman) with a different upper limit of normal (ULN) than that previously used (Centaur), the instructions for further assessments of subjects with an absolute level of cardiac troponin of >0.5 ng/mL are now presented as the difference between the most recent value of cardiac troponin and the ULN, and not an absolute value. The current criterion is equivalent to the previous one, given that the absolute number of >0.5 ng/mL is 17× the ULN of the previous assay and the absolute number of >1.0 ng/mL is 33× the ULN. In Section 8.4.3.1, added the following paragraphs: • A male family member or healthcare provider who has been exposed to the study intervention by skin, mucosal contact, or ingestion then exposes his female partner prior to or around the time of conception. • A female household member of a study subject reports that she is pregnant and has been exposed through viral shedding by a study subject within 2 months after

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		dosing, and a month prior to or during pregnancy. • Rationale: For consistency with the Phase 3 study. In Section 13.1, updated "primary completion date" to "end of trial." • Rationale: Clarification based on the definition of primary completion date. In Appendix 1, added statement to indicate summary statistics will be generated for a cohort of at least 3 subjects. • Rationale: To provide clarity as to when a statistical summary would be generated for the sirolimus cohort. Appendix 1 (Changes that were made via the PACL dated 24-Nov-2021): Added a footnote to the Sirolimus SoA to indicate that a phone communication between the site staff and the subject and/or caregiver will be conducted to remind the subject to start taking the sirolimus if dispensed at baseline. • Rationale: To add clarity. Removed "Dosing + 24 Hour Monitoring + Initial Follow-Up" from the Visit 3 header in the Sirolimus SoA. • Rationale: To add clarity. Visit 3 in the main SoA includes days 1, 2, 4, 7, and 10 which applies to dosing, monitoring, and follow-up after PF-06939926 administration. The activity to be conducted during Visit 3 for the sirolimus cohort is to obtain the trough level on Day 4. As such, text referring to dosing of PF-06939926 and 24 hour monitoring are not relevant and could be misleading.

This amendment incorporates all revisions to date, including amendments made at the request of country health authorities and institutional review boards (IRBs)/ethics committees (ECs).

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SCHEDULE OF ACTIVITIES

The schedule of activities table provides an overview of the protocol visits and procedures. Refer to the STUDY PROCEDURES BY VISIT and STUDY ASSESSMENTS sections of the protocol for detailed information on each procedure and assessment required for compliance with the protocol.

The investigator may schedule visits (unplanned visits) in addition to those listed on the Schedule of Activities table in order to conduct evaluations or assessments required to protect the well-being of the subject. For the sirolimus cohort, in addition to the Schedule of Activities below, see Appendix 1.

Period	**Screening**	**Baseline**	**Treatment**	**1st Year Follow-up**											**Long-Term Follow-up and/or Early Termination**
Visit Number	**Visit 1[a,i] Screening**	**Visit 2[a,g] Baseline**	**Visit 3[b] Dosing + 24 Hour Monitor-ing + initial follow-up**	**Visit 4[b] Week 2**	**Remote[c] Visit 5 Week 3**	**Visit 6 Week 4**	**Remote[c] Visit 7 Week 6**	**Visit 8[a] Week 8**	**Remote[c] Visit 9 Week 10**	**Visit 10 Week 12**	**Remote[c] Visits 11.1, 11.2, 11.3 Weeks 15, 19, 22**	**Visit 12[a] Week 26**	**Remote[c] Visits 12.1 and 12.2 Weeks 32, 39**	**Visit 13[a] Week 52**	**Remote[f] visits 13.1, 14.1, 15.1, 16.1; Years 1.5, 2.5, 3.5, 4.5 Visits 14-17[a] Years 2-5 /Early Termination (ET)**
Visit Window	**Day -196 to Day -17**	**Day -16[g]** (-12 to +2 days)	**Day 1, Day 2 (Discharge), Days 4[j], 7, 10 (±1 day)**	**Day 14** (±1 day)	**Day 21** (±3 days)	**Day 30** (±3 days)	**Day 45** (±3 days)	**Day 60** (±3 days)	**Day 75** (±3 days)	**Day 90** (±3 days)	**Days 106, 135, 155** (±7 days)	**Day 180** (±7 days)	**Days 225, 270** (±7 days)	**Day 360** (±7 days)	**Remote Days 545, 910, 1275, 1640; Visit Days 730, 1095, 1460, 1825** (±30 days)/**ET**
Informed consent/assent[d]	X														
Demography	X														
Medical and Medication History[e]	X														

Period	**Screening**	**Baseline**	**Treatment**	**1st Year Follow-up**											**Long-Term Follow-up and/or Early Termination**
Visit Number	**Visit 1[a,i] Screening**	**Visit 2[a,g] Baseline**	**Visit 3[b] Dosing + 24 Hour Monitor-ing + initial follow-up**	**Visit 4[b] Week 2**	**Remote[c] Visit 5 Week 3**	**Visit 6 Week 4**	**Remote[c] Visit 7 Week 6**	**Visit 8[a] Week 8**	**Remote[c] Visit 9 Week 10**	**Visit 10 Week 12**	**Remote[c] Visits 11.1, 11.2, 11.3 Weeks 15, 19, 22**	**Visit 12[a] Week 26**	**Remote[c] Visits 12.1 and 12.2 Weeks 32, 39**	**Visit 13[a] Week 52**	**Remote[t] visits 13.1, 14.1, 15.1, 16.1; Years 1.5, 2.5, 3.5, 4.5 Visits 14-17[a] Years 2-5 /Early Termination (ET)**
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Inclusion/ Exclusion Review	X	X - *NAb only*[i]													
Physical and Neurological Examination	X	X		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]	X[f] – *annual and ET only*
Height and Weight	X	X[g]						X				X		X	X – *annual and ET only*
Vital Signs[h]	X	X	X[h]	X[h]		X[h]		X		X		X		X	X – *annual and ET only*
Triplicate ECG	X		X[v] – *Day 1 only*	X		X		X				X		X	X – *annual and ET only*
Laboratory Assessments															
Blood samples[x]															

Period	**Screening**	**Baseline**	**Treatment**	**1st Year Follow-up**											**Long-Term Follow-up and/or Early Termination**
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CCI															
Clinical safety[k]	X[k,l]	X[l]	X[j,k] –*also includes local labs Days 5-9*	X[k]	X[k]	X[k]	X[k]	X[k]	X	X	X	X	X	X	X[t]
Exploratory biomarkers (serum, plasma, RNA)	X[x]	X[x]	X[x] – *plasma and serum on Days 4, 7, 10*	X[x]– *plasma and serum*	X[x]- *plasma and serum*			X[x]				X		X	X[t]
CCI															

Period	**Screening**	**Baseline**	**Treatment**	**1st Year Follow-up**											**Long-Term Follow-up and/or Early Termination**
Visit Number	**Visit 1[a,i] Screening**	**Visit 2[a,g] Baseline**	**Visit 3[b] Dosing + 24 Hour Monitor-ing + initial follow-up**	**Visit 4[b] Week 2**	**Remote[c] Visit 5 Week 3**	**Visit 6 Week 4**	**Remote[c] Visit 7 Week 6**	**Visit 8[a] Week 8**	**Remote[c] Visit 9 Week 10**	**Visit 10 Week 12**	**Remote[c] Visits 11.1, 11.2, 11.3 Weeks 15, 19, 22**	**Visit 12[a] Week 26**	**Remote[c] Visits 12.1 and 12.2 Weeks 32, 39**	**Visit 13[a] Week 52**	**Remote[t] visits 13.1, 14.1, 15.1, 16.1; Years 1.5, 2.5, 3.5, 4.5 Visits 14-17[a] Years 2-5 /Early Termination (ET)**
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Urine samples															
CCI [redacted]															
Tissue samples															
Muscle biopsy[n]		X[n] *#1*						*X[o] #2a*		*X[o] #2b*		*X[o] #3a*		*X[o] #3b*	X-*ET only[o]*
Various															
CCI [redacted]															
Imaging Assessments[a,q]															
CCI [redacted]															

Period	**Screening**	**Baseline**	**Treatment**	**1st Year Follow-up**											**Long-Term Follow-up and/or Early Termination**
Visit Number	**Visit 1[a,i] Screening**	**Visit 2[a,g] Baseline**	**Visit 3[b] Dosing + 24 Hour Monitor-ing + initial follow-up**	**Visit 4[b] Week 2**	**Remote[c] Visit 5 Week 3**	**Visit 6 Week 4**	**Remote[c] Visit 7 Week 6**	**Visit 8[a] Week 8**	**Remote[c] Visit 9 Week 10**	**Visit 10 Week 12**	**Remote[c] Visits 11.1, 11.2, 11.3 Weeks 15, 19, 22**	**Visit 12[a] Week 26**	**Remote[c] Visits 12.1 and 12.2 Weeks 32, 39**	**Visit 13[a] Week 52**	**Remote[t] visits 13.1, 14.1, 15.1, 16.1; Years 1.5, 2.5, 3.5, 4.5 Visits 14-17[a] Years 2-5 /Early Termination (ET)**
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Cardiac MRI[p]	X													X	X – *annual and ET only*
Functional Assessments[a,q]															
CCI [redacted]	[redacted]	[redacted]								[redacted]		[redacted]	[redacted]	[redacted]	[redacted]
Questionnaires[r]															
C-SSRS	X	X		X								X		X	X – *annual and ET only*
CCI [redacted]		[redacted]								[redacted]		[redacted]		[redacted]	[redacted]

Period	**Screening**	**Baseline**	**Treatment**	**1st Year Follow-up**											**Long-Term Follow-up and/or Early Termination**
Visit Number	**Visit 1[a,i] Screening**	**Visit 2[a,g] Baseline**	**Visit 3[b] Dosing + 24 Hour Monitoring + initial follow-up**	**Visit 4[b] Week 2**	**Remote[c] Visit 5 Week 3**	**Visit 6 Week 4**	**Remote[c] Visit 7 Week 6**	**Visit 8[a] Week 8**	**Remote[c] Visit 9 Week 10**	**Visit 10 Week 12**	**Remote[c] Visits 11.1, 11.2, 11.3 Weeks 15, 19, 22**	**Visit 12[a] Week 26**	**Remote[c] Visits 12.1 and 12.2 Weeks 32, 39**	**Visit 13[a] Week 52**	**Remote[t] visits 13.1, 14.1, 15.1, 16.1; Years 1.5, 2.5, 3.5, 4.5 Visits 14-17[a] Years 2-5 /Early Termination (ET)**
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Subject dosing diary[u]			X – *starts Day 2*	→	→	X	→	X	→	X					
Enrollment															
Registration		X[g]													
Study Treatment															
Pre-/post-dose concomitant glucocorticoid medication[s]			X	→	→	→	→	→	→	X					
Study drug administration[b]			X – *Day 1 only*												
Meningococcal Vaccine[a]	X	X													
Ongoing monitoring															
Wellness check-in					X		X		X		X	X	X	X	X[t]
Concomitant med monitoring		X[aa]	→	→	→	→	→	→	→	→	→	→	→	→	→

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AE, and infusion site monitoring[b]	X	→	→	→	→	→	→	→	→	→	→	→	→	→	→

Abbreviations/Acronyms: → = continuous monitoring/event; CCI [redacted] ADA=anti-drug antibody; AE=Adverse events; BP=Blood pressure; C-SSRS=Columbia Suicide Severity Rating Scale, ECG = electrocardiogram; ELISpot= Enzyme-Linked ImmunoSpot; CCI [redacted] CCI [redacted] EQ-5D-5L= EuroQuol 5 Dimensions-5 Levels; ET=early termination; GLDH=glutamate dehydrogenase; HR=heart rate; med=medication(s); MRI=magnetic resonance imaging; NAb=neutralizing antibodies; Prep D1.5=whole blood for deoxyribonucleic acid (DNA); NSAA=North Star Ambulatory Assessment; CCI [redacted] RNA=ribonucleic acid; temp=temperature; CCI [redacted]

a. Subjects may be asked to return to the investigational site 2 or 3 times for visits at which biopsies, imaging, and/or functional assessments are required. Meningococcal Vaccine: Subjects who have no contraindications and who have not previously received a MenACWY vaccination; or whose last vaccination at the time of the Screening visit (Visit 1) is outside the time period of active coverage specified by the vaccine manufacturer (Visit 1) must receive at least 1 dose of MenACWY vaccine as early as possible in the Screening Period and not later than 30 days before study drug administration (see Section 5.7.1). Subjects must also receive MenB vaccination if indicated by national vaccination guidelines. In addition, local eculizumab prescribing information and other requirements must also be followed (see Section 5.8).

b. Subjects will be admitted to the investigational site to receive a single intravenous infusion of PF-06939926 administered over approximately 2 to 4 hours (+30 minutes including flush) on the Dosing visit (ie, Day 1), as detailed in the Investigational Product Manual, and will be monitored for at least 24 hours following study drug administration (ie, Day 2), or longer if deemed necessary. If adverse events (AEs) possibly related to PF-06939926 are observed, subjects should not be discharged until the events have resolved or can be managed by the subject and/or caregiver (eg, oral antiemetics are confirmed to effectively stop vomiting events). Upon discharge,

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subjects should stay near the investigational site to enable easy follow-up in the event of any emergent AEs through the Day 14 (Week 2) visit, or longer if clinically indicated. Subjects will return to the investigational site on Days 4, 7, 10 (±1 day) for biospecimen collection, as well as assessment of any new or worsening adverse events, changes to concomitant medications, and confirmation of adequate fluid intake and urine output through Week 4. Blood and urine samples will also be obtained for local testing on Days 5-9, or longer, as clinically indicated. Subjects will be monitored for signs of infusion site reactions from the start of treatment of PF-06939926 and then at the in-person visits through Week 8 (Day 60 ±3 days), and/or as clinically indicated.

c. Unless clinical concern and/or subject preference warrants in-person visit, remote visits may be performed at Weeks 3, 6, 10, 15, 19, 22, 32 and 39 (ie, Visits 5, 7, 9, 11.1, 11.2, 11.3, 12.1, and 12.2, respectively), which would include blood, saliva, and urine collection at the subject's home coordinated by local phlebotomist, as well as phone or email communication between site staff and subject and/or caregiver to discuss any adverse events and/or changes to concomitant medications. Adequate fluid intake and output may also be remotely confirmed during the Week 3 communication.

d. Informed consent must be provided by the subject, depending on age, or legally authorized representative (eg, parent or legal guardian). Subjects who are minors may also be required to provide assent in compliance with local regulations and institutional review board (IRB) requirements.

e. Medical history will also include confirmation by genetic testing of the diagnosis of Duchenne muscular dystrophy (DMD). Medication history must include at least 6 months of glucocorticoids with 3 months on a stable, daily dose relative to the Baseline visit.

f. Only brief physical and neurological examinations will be performed post-baseline unless safety concerns warrant full examination.

g. Amount of study drug shipped to site will occur once the subject is registered (ie, enrolled) following confirmation of eligibility and measurement of body weight at Baseline. The Baseline visit must occur at least 2-4 weeks (14-28 calendar days) prior to the planned Dosing visit.

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h. Vital signs will always consist of blood pressure, respiration rate, and heart rate, but body temperature will also be obtained with these measurements at the following timepoints: within 30 minutes before and approximately 30 minutes, 1, 2, 4, 8, and 24 hours after the start of the infusion, and on Days 4, 7, 10, 14 and 30. Body temperature at additional timepoints may be collected as clinically indicated.

k. Clinical safety labs are described in Table 1. Additional safety labs on Days 1 (pre-dose) through Week 8 will include CCI and will continue to be collected until within normal limits or return to baseline.

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l. Screening labs will include the safety labs (Table 1) and the following: DMD genetic testing (if required to confirm DMD diagnosis), anti-hepatitis A immunoglobulin M, anti-hepatitis B surface antigen, hepatitis C antibody, antinuclear antibody (ANA), total immunoglobulin G, and international normalized ratio. If time between Screening labs and the planned Baseline visit exceeds 12 weeks (84 days), then all Screening labs except for DMD genetic testing should be repeated and eligibility (re)confirmed prior to enrolling the subject.

m. Urinalysis may be performed at additional planned or unscheduled visits if clinically indicated.

n. Muscle biopsies (eg, open, needle) will be obtained following administration of an anesthetic (eg, regional block or under general anesthesia) according to institutional standard practice and will occur preferably after any imaging and functional assessments scheduled for the same visit. If baseline muscle biopsies have been obtained outside this study and (1) can be shipped to the sponsor's designated lab for use in this study and (2) are deemed by the sponsor to be of sufficient quantity and quality, these may replace the need for an additional baseline biopsy.

o. Biopsies will be collected from a single subject at a maximum of 3 visits during the study at the following timepoints: (1) Baseline, (2) ***either*** Week 8 (Day 60) ***or*** Week 12 (Day 90), and (3) ***either*** Week 26 (Day 180) ***or*** Week 52 (Day 360). If a subject withdraws participation in the study prior to completion of the 1st Year Follow-up, a biopsy at the Early Termination visit may only be obtained if subject has not yet completed all 3 procedures.

p. Cardiac MRI should be performed *after* thigh and upper limb MRI and/or on separate days within the required visit window, if preferred by site and/or subject. Cardiac MRI already obtained within 3 months prior to the Screening visit may be deemed adequate for confirming subject eligibility (see Section 6.1). If cardiac MRI is attempted, but is unable to be evaluated (eg, due to young subject's inability to remain still during the scan), it may either be repeated or substituted by a locally-read echocardiogram.

Period	Screening	Baseline	Treatment	1st Year Follow-up											Long-Term Follow-up and/or Early Termination
Visit Number	**Visit 1[a,i] Screening**	**Visit 2[a,g] Baseline**	**Visit 3[b] Dosing + 24 Hour Monitor-ing + initial follow-up**	**Visit 4[b] Week 2**	**Remote[c] Visit 5 Week 3**	**Visit 6 Week 4**	**Remote[c] Visit 7 Week 6**	**Visit 8[a] Week 8**	**Remote[c] Visit 9 Week 10**	**Visit 10 Week 12**	**Remote[c] Visits 11.1, 11.2, 11.3 Weeks 15, 19, 22**	**Visit 12[a] Week 26**	**Remote[c] Visits 12.1 and 12.2 Weeks 32, 39**	**Visit 13[a] Week 52**	**Remote[t] visits 13.1, 14.1, 15.1, 16.1; Years 1.5, 2.5, 3.5, 4.5 Visits 14-17[a] Years 2-5 /Early Termination (ET)**
Visit Window	**Day -196 to Day -17**	**Day -16[g]** (-12 to +2 days)	**Day 1, Day 2 (Discharge), Days 4[j], 7, 10 (±1 day)**	**Day 14** (±1 day)	**Day 21** (±3 days)	**Day 30** (±3 days)	**Day 45** (±3 days)	**Day 60** (±3 days)	**Day 75** (±3 days)	**Day 90** (±3 days)	**Days 106, 135, 155** (±7 days)	**Day 180** (±7 days)	**Days 225, 270** (±7 days)	**Day 360** (±7 days)	**Remote Days 545, 910, 1275, 1640; Visit Days 730, 1095, 1460, 1825** (±30 days)/**ET**

[redacted]

r. As described in Section 7.7, depending on factors such as the subject's age, ambulatory status at enrollment, and at the discretion of the investigator and caregiver, questionnaires will be completed by the subjects themselves and/or by the legal caregiver on behalf of the subject. Legal caregivers will also be asked to complete self-assessments. As per Section 7.2.8, the Columbia Suicide Severity Rating Scale (C-SSRS) will be completed by the legal caregiver on behalf of the subject, or by the subject himself depending on his age at Screening (ie, < or ≥12 years of age, respectively).

s. Between one and four hours prior to infusion of study drug, subjects will receive ⩾2 mg/kg intravenous methylprednisolone. Subjects will replace pre-study daily glucocorticoids with oral prednisone or prednisolone at increased daily dose levels for the first 3 months post-dose of study drug, after which, so long as there is no immune response or other clinical indication, subjects may return to their pre-study daily glucocorticoid regimen for the remainder of the first year of post-treatment (see Section 5).

[redacted]

u. Subjects or caregivers on behalf of subjects may be required to complete dosing diaries to ensure compliance with the increased daily dose levels of oral prednisone or prednisolone required for at least the first 3 months post-dose of study drug. If diary is not used, compliance with the daily glucocorticoid regimen is expected to be

Period	Screening	Baseline	Treatment	1st Year Follow-up											Long-Term Follow-up and/or Early Termination
Visit Number	**Visit 1[a,i] Screening**	**Visit 2[a,g] Baseline**	**Visit 3[b] Dosing + 24 Hour Monitor-ing + initial follow-up**	**Visit 4[b] Week 2**	**Remote[c] Visit 5 Week 3**	**Visit 6 Week 4**	**Remote[c] Visit 7 Week 6**	**Visit 8[a] Week 8**	**Remote[c] Visit 9 Week 10**	**Visit 10 Week 12**	**Remote[c] Visits 11.1, 11.2, 11.3 Weeks 15, 19, 22**	**Visit 12[a] Week 26**	**Remote[c] Visits 12.1 and 12.2 Weeks 32, 39**	**Visit 13[a] Week 52**	**Remote[t] visits 13.1, 14.1, 15.1, 16.1; Years 1.5, 2.5, 3.5, 4.5 Visits 14-17[a] Years 2-5 /Early Termination (ET)**
Visit Window	**Day -196 to Day -17**	**Day -16[g]** (-12 to +2 days)	**Day 1, Day 2 (Discharge), Days 4[j], 7, 10 (±1 day)**	**Day 14** (±1 day)	**Day 21** (±3 days)	**Day 30** (±3 days)	**Day 45** (±3 days)	**Day 60** (±3 days)	**Day 75** (±3 days)	**Day 90** (±3 days)	**Days 106, 135, 155** (±7 days)	**Day 180** (±7 days)	**Days 225, 270** (±7 days)	**Day 360** (±7 days)	**Remote Days 545, 910, 1275, 1640; Visit Days 730, 1095, 1460, 1825** (±30 days)/**ET**

reviewed with the subject and/or caregiver and documented following all in-clinic and remote visits (ie, wellness check-ins) through at least the first year following PF-06939926 administration.

v. On Day 1, triplicate ECGs will be performed approximately one hour prior to infusion of study drug, as well as upon completion of the infusion (+30 minutes) and as clinically indicated.

w. Activity monitors may be placed on the subject's wrist and/or ankle prior to the other functional assessments and will be worn continuously for the subsequent 2 weeks, except for remote visit at Week 39 (Day 270±7 days), in which case activity monitors will be sent directly to the subject's home for 2-week continuous wear in free-living conditions. No functional tests are expected to be performed at Week 39. Certain functional assessments will be avoided for subjects who are or become non-ambulatory (ie, inability to walk 10 meters unassisted), as described in Section 7.5.

Period	Screening	Baseline	Treatment	1st Year Follow-up											Long-Term Follow-up and/or Early Termination
Visit Number	**Visit 1[a,i] Screening**	**Visit 2[a,g] Baseline**	**Visit 3[b] Dosing + 24 Hour Monitor-ing + initial follow-up**	**Visit 4[b] Week 2**	**Remote[c] Visit 5 Week 3**	**Visit 6 Week 4**	**Remote[c] Visit 7 Week 6**	**Visit 8[a] Week 8**	**Remote[c] Visit 9 Week 10**	**Visit 10 Week 12**	**Remote[c] Visits 11.1, 11.2, 11.3 Weeks 15, 19, 22**	**Visit 12[a] Week 26**	**Remote[c] Visits 12.1 and 12.2 Weeks 32, 39**	**Visit 13[a] Week 52**	**Remote[t] visits 13.1, 14.1, 15.1, 16.1; Years 1.5, 2.5, 3.5, 4.5 Visits 14-17[a] Years 2-5 /Early Termination (ET)**
Visit Window	**Day -196 to Day -17**	**Day -16[g]** (-12 to +2 days)	**Day 1, Day 2 (Discharge), Days 4[j], 7, 10 (±1 day)**	**Day 14** (±1 day)	**Day 21** (±3 days)	**Day 30** (±3 days)	**Day 45** (±3 days)	**Day 60** (±3 days)	**Day 75** (±3 days)	**Day 90** (±3 days)	**Days 106, 135, 155** (±7 days)	**Day 180** (±7 days)	**Days 225, 270** (±7 days)	**Day 360** (±7 days)	**Remote Days 545, 910, 1275, 1640; Visit Days 730, 1095, 1460, 1825** (±30 days)/**ET**

z. At Screening, only the functional assessments to confirm eligibility based on a subject's ambulatory status (ie, Ambulatory status for all subjects, but only NSAA for ambulatory subjects, defined as ability to walk 10 meters unassisted and pulmonary function tests and Performance of Upper Limb (PUL), version 2.0 for non-ambulatory subjects) will be performed (see Section 6.1). If time between Screening functional assessments and the planned Baseline visit exceeds 12 weeks (84 days), then these should be repeated and eligibility (re)confirmed prior to enrolling the subject. All functional assessments, including activity monitoring, will be performed at Baseline and the visits specified.

aa. Subjects should receive meningococcal vaccinations at least 30 days prior to administration of PF-06939926 (ie, Baseline visit). Alternatively, (eg, in the case of allergy) prophylactic antibiotics for meningococcus may be provided simultaneously with eculizumab, if administered (see Section 5.8).

1. INTRODUCTION

1.1. Mechanism of Action/Indication

The investigational product, PF-06939926, is a gene therapy viral construct, AAV9.hCK.opti-DysΔ3978.spolyA, that consists of a recombinant adeno-associated virus serotype 9 (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, hybrid creatine kinase (hCK).[1] This agent is in development for the treatment of Duchenne muscular dystrophy (DMD), an X -linked neuromuscular disease primarily affecting boys as described in Section 1.2.1.

Protocol C3391001 is the first-in-human (FIH)/first-in-patient (FIP) study with PF-06939926. This study aims to evaluate the safety and tolerability following a single dose of PF-06939926 in ambulatory and non-ambulatory subjects 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.

Complete information for this compound may be found in the Single Reference Safety Document, which for this study will be the Investigator's Brochure (IB).

1.2. Background and Rationale

1.2.1. Duchenne Muscular Dystrophy

Duchenne muscular dystrophy (DMD) is the most frequently inherited pediatric neuromuscular disease and predominantly affects boys. It is an X-linked muscular dystrophy caused by a mutation at Xp21 in the gene coding for the protein dystrophin[2] which is absent from the muscle of boys with DMD. The most common forms of mutations that cause DMD are large deletion mutations of one or more exons (68%). However, large duplication mutations (11%), point mutations (11%), and small insertion/deletion mutations (7%) also occur.[3] These gene mutations often lead to a frame shift mutation (ie, disruption of the reading frame) or generate a premature stop codon (ie, out-of-frame) and result in a lack of dystrophin protein. The majority of in-frame deletions result in a milder form of the disease, Becker muscular dystrophy (BMD), in which patients express a truncated, partially functional dystrophin.

The lack of the dystrophin protein in DMD boys leads to skeletal muscle, and ultimately heart and respiratory muscle, degeneration causing premature death.[4] Progressive weakness and muscle atrophy begin in childhood, starting in the lower legs and pelvis before spreading into the upper arms. Other symptoms include loss of some reflexes, a waddling gait, frequent falls, difficulty when rising from a sitting or lying position or when climbing stairs, changes to overall posture, impaired breathing, and cardiomyopathy. Many children are unable to run rapidly or to jump. The atrophied muscles, in particular the calf muscles (and, less commonly, muscles in the buttocks, shoulders, and arms), may be enlarged by an accumulation of fat and connective tissue, causing them to look larger and healthier than they actually are (called pseudohypertrophy). Bone thinning and scoliosis are common. Ultimately, a wheelchair becomes necessary, in most cases between 12 to 15 years of age.[5]

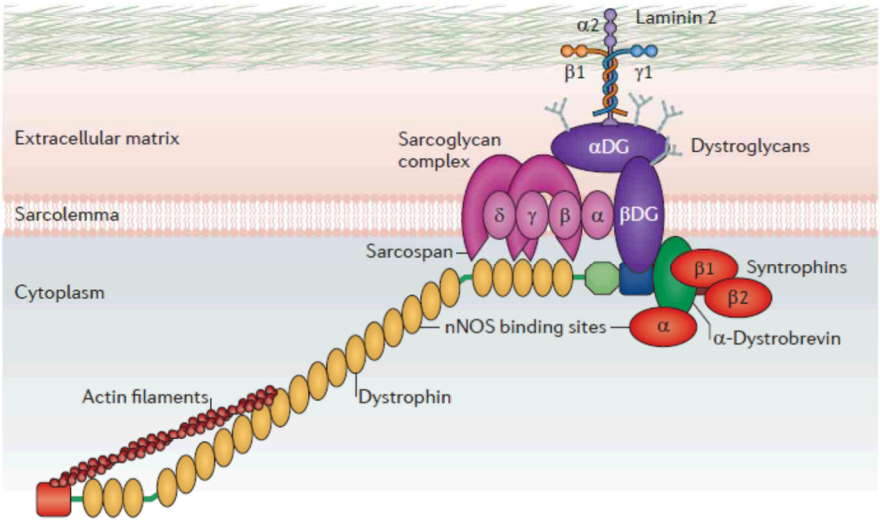
There is no specific approved treatment that can stop or reverse the progression of DMD. There have been three recent US drug approvals for DMD. EXONDYS 51™ (eteplirsen) was recently approved based on a surrogate endpoint of increased dystrophin protein production, but is only indicated for DMD patients that have an exon 51 skip amenable mutation which occurs in about 13% of DMD patients.[6] VYONDYS 53™ (golodirsen) is intended to allow the transcription process to skip over exons that contain mutations ('exon skipping') in the region of exon 53,[7] which occurs in approximately 8% of DMD patients. EMFLAZA™ (deflazacort) was approved for the treatment of DMD patients 5 years of age and older based on improved muscle strength compared to placebo. Disease management consists primarily of supportive care which ranges from physical therapy to maximize function and minimize muscle contractures, to orthotics, tendon release surgery (which provides temporary benefit as contractures will redevelop), use of wheelchair (usually by age 12), surgical correction of scoliosis[8] and use of respiratory care including various forms of assisted ventilation (including tracheotomy). Respiratory infections may be treated with antibiotics. Death from pneumonia or cardiac involvement in late teens or early twenties is commonly observed in these patients.[9,10]

A key therapeutic goal is to slow or prevent muscle degeneration in these patients. Only limited approaches, such as chronic glucocorticoid use, are currently available, but glucocorticoid use is associated with well known adverse events such as weight gain and broad hypothalamic pituitary adrenal axis effects.

1.2.2. Dystrophin Role in Muscle

Dystrophin is a cytoplasmic protein encoded by the *DMD* gene, which links cytoskeletal actin filaments to membrane proteins. Normally, the dystrophin protein, located primarily in skeletal and cardiac muscles, with smaller amounts expressed in the brain, acts as a shock absorber during muscle fiber contraction by linking the actin of the contractile apparatus to the layer of connective tissue that surrounds each muscle fiber (Figure 1).[11,12,13]

Figure 1. The Dystrophin-associated Protein (DAP) Complex[12]



Dystrophin acts as an important link between the internal cytoskeleton and the extracellular matrix. αDG, α-dystroglycan; βDG, β-dystroglycan.

The *DMD* gene is particularly complex and contains at least eight independent and tissue-specific promoters. The full-length dystrophin isoform for instance, is transcribed from three independently regulated promoters.[14] Apart from the full-length dystrophin isoforms (427 kDa), five additional isoforms exist due to splicing of the dystrophin ribonucleic acid (RNA). These additional isoforms are named according to their respective molecular weights: Dp260 (predominantly expressed in the retina), Dp140 (expressed in central nervous system [CNS] and kidney), Dp116 (detected in the peripheral nervous system), Dp71 (expressed in most tissues but not in muscle), and Dp40 (expressed in the brain).[15-19]

In muscle, dystrophin is localized to the cytoplasmic face of the sarcolemma membrane. Full-length dystrophin (Dp427) consists of an N-terminal actin-binding domain, a central large rod-like domain composed of spectrin-like repeats, and a cysteine rich (CR) C-terminus that is connected to the dystrophin-associated glycoprotein complex (DGC), a large protein complex that forms a critical link between the cytoskeleton and the extra-cellular matrix.[20] In addition to dystrophin, this complex consists of dystroglycan (α and β), sarcoglycan (α, β, γ and δ), sarcospan, syntrophin (α1, β1, β2, γ1, γ2), and α-dystrobrevin.[21] Alpha and beta dystroglycan (dystrophin-associated glycoprotein) bind laminin, a component of the basal lamina, and dystrophin respectively, thereby providing a link between the cytoskeleton and the extracellular matrix. Sarcoglycan is the second transmembrane component of the DGC. On the cytoplasmic side, the sarcolemmal DGC, through dystrophin, interacts with syntrophin and α-dystrobrevin. These latter proteins recruit scaffolding proteins onto which signaling proteins and ion channels are anchored in the membrane.[22] In this way, the DGC both provides a physical and functional connection between the internal and external environment of the muscle cell.

Due to its pivotal role in the structure of muscle cells, dystrophin restoration, or replacement via gene transfer, requires generation of some form of the protein able to reassemble the DGC and support a mechanically strong link between the extra-cellular matrix and the cytoskeleton.[23]

1.2.3. Gene Therapy for the Treatment of DMD

The muscular dystrophies, and in particular DMD, are attractive candidates for gene transfer, as they arise from single-gene mutations. While many advances have occurred, the development of an effective gene therapy for DMD still faces significant challenges. These include in particular (i) determining the optimal mode of gene delivery in order to reach all targeted muscle cells, (ii) overcoming the packaging requirements of AAV and (iii) overcoming the immune challenges linked to the reintroduction of a gene that may be recognized as foreign by the immune system of DMD patients. The optimized vector, PF-06939926, has been designed to overcome such challenges for the treatment of DMD.

In brief, recombinant AAV (rAAV) are based on a non-pathogenic, non-integrative, replication deficient member of the parvovirus family. Recombinant AAV (rAAV) vectors are ideal candidates for muscle-directed gene therapy because of (i) their ability to target skeletal and cardiac muscle, (ii) the long-term persistence of the vector genome in transduced cells, and (iii) their lack of immunotoxicity.[24] Gene delivery using a variety of AAV serotypes has been investigated in clinical trials for diseases including hemophilia B, alpha-1 antitrypsin deficiency, lipoprotein lipase deficiency, Pompe disease, heart failure, and several muscular dystrophies, including DMD (www.clinicaltrials.gov). Safety and long-term transgene expression has been reported in several of these studies, with no AAV-related severe adverse events.[25] Finally, the AAV9 capsid has been shown to have a strong and efficient tropism for both skeletal and cardiac muscle, resulting in transduction throughout the body after systemic administration in several preclinical studies.[26-29]

As mentioned, a major hurdle in the use of rAAV vectors in DMD gene therapy is their packaging capacity, which is about 4.7 kb, while the size of the dystrophin complimentary deoxyribonucleic acid (cDNA) is about 14 kb. To overcome this challenge, a number of groups have developed partially deleted (3.7–4.5 kb) but highly functional dystrophin genes that can be successfully packaged inside rAAV vectors.[24,30,31] Dystrophin mini-or micro-genes have been designed on the basis of the mutant form of the gene carried in a mildly affected patient with BMD. This form has a large in-frame rod domain deletion and fits into AAV vectors even when muscle-specific promoters are incorporated.[24,31,32]

The truncated dystrophin proteins, called mini or micro-dystrophins, encoded by the rAAV vectors, have been shown to improve the dystrophic phenotype in animal models of DMD.[24,26,32-35] Restoration of the dystrophin-associated protein complex, stabilization of muscle degeneration, and improvements in muscle function have been demonstrated following delivery of truncated dystrophin genes at different stages of disease progression in the murine model for DMD (mdx mice).[36,37,38] However, the ability of these minimized dystrophin proteins to protect muscle from contraction-induced injury is linked both to the structural elements included and to the expression levels of the therapeutic protein.[34]

The first clinical trial of muscle-directed delivery of DysΔ3990, under the control of cytomegalovirus (CMV) promoter and packaged in a chimeric AAV capsid, AAV2.5, was conducted in DMD patients.[39] This placebo-controlled Phase 1 trial involved the unilateral injection of 6E+11 or 3E+12 vector genomes (vg) to the biceps muscle of six male subjects (5 to 11 years old) with DMD. Saline or empty capsid was injected into the contralateral arm. All subjects received 2 mg/kg intravenous (IV) methylprednisolone four hours pre- and 24 and 48 hours post-vector injection to reduce the potential for local inflammation. The treatment was well-tolerated, with no acute or chronic product-related adverse events reported. Notably, no elevations from baseline of creatine kinase, alkaline phosphatase or liver enzymes were observed. Transgene DNA was detected in muscle biopsies of the injected, but not contralateral, arm of all six subjects. However, limited mini-dystrophin-positive myofibers were detected in only two of the six treated subjects, one each at the low and high dose. Mini-dystrophin specific T-cells were detected in three of the six subjects.[40] The lack of mini-dystrophin expression in the treated subjects could be explained by many factors, including loss of transduced cells due to disease-associated inflammation or a T-cell mediated response primed either by the expressed mini-dystrophin protein or prior endogenous revertant dystrophin epitopes. Alternative explanations for low transgene expression include silencing of the CMV promoter or low transduction efficiency of AAV2.5 either due to pre-existing neutralizing antibodies (NAb) or low capsid tropism in human muscle tissue.

The intramuscular (IM) route of administration (ROA) examined in this first mini-dystrophin gene transfer study has several limitations for the treatment of DMD. The ROA has been shown to influence the immune response to rAAV vector encoded transgene expression in non-human primate muscle, with immune-mediated destruction of transduced muscle observed after direct, but not regional IV (isolated limb perfusion), rAAV delivery.[41] Therefore, the impact of the IM ROA on the low level of transgene expression cannot be ruled out, especially since the disease progresses and muscle destruction continues. Furthermore, successful treatment of DMD will require global correction of skeletal and cardiac muscle that cannot be achieved by IM administration. While it has been shown that widespread vector distribution to the injected muscle can be achieved after isolated limb perfusion but not IM delivery, global transduction of skeletal and cardiac muscle would be achievable following systemic delivery of a rAAV vector capable of efficiently transducing both skeletal and cardiac muscle.[42] Thus, the vector construct of PF-06939926 used in this Phase 1b DMD clinical trial described above has been further optimized to overcome gene therapy challenges for the treatment of DMD.

1.3. Immunogenicity Risk Assessment

1.3.1. Nonclinical Immunogenicity

During the evaluation of systemic delivery of PF-06939926 at various doses in the DMD rat model, human mini-dystrophin was seen by the host immune system; however, the humoral response detected against mini-dystrophin and the AAV9 capsid had no impact on the therapeutic efficacy of the PF-06939926 3- and 6-months post-infusion. In addition, there was no correlation between the lack of a cellular immune response to the transgene and the humoral response observed. The association of systemic delivery of an AAV encoding for a

foreign polypeptide with the destruction of the transduced cells by a detectable cellular immunity is unclear.[67] The route of administration is believed to be an important component of immune response outcome with the IV route being the least immunogenic, at least in animal models, including rodents, dogs and non-human primates.

1.3.2. Nonclinical Pharmacodynamics

A dose-finding study was conducted in DMD rats and demonstrated a dose-dependent increase the percentage of fibers expressing mini-dystrophin as well as a decrease in fibrosis. Additionally, increasing doses of vector resulted in a dose-dependent decrease in fatigue as measured by repeat grip testing in the DMD rats. The data from this dose range study in DMD rats suggest that 1E+14 vg/kg is the minimally effective dose. CCI CCI CCI Additionally, expression of the transgene in muscle tissues and preservation of the functional responses persisted 6 months post-dose.

1.4. Study Design Rationale

This FIH/FIP study will evaluate the safety and tolerability of a single administration of up to 3 dose levels of PF-06939926 in ambulatory and non-ambulatory subjects diagnosed with DMD with an absence of neutralizing antibodies (NAb) to AAV9. Up to approximately 22 subjects may be enrolled to enable a robust understanding of the safety profile of PF-06939926. Additionally, this study will provide information on this gene transfer approach by assessing the expression and distribution of mini-dystrophin protein in the muscle of subjects with DMD. The study will be conducted in two phases: (1) the 1st Year Follow-up to primarily assess safety, as well as expression and distribution of mini-dystrophin at 2 or 3 and 6 or 12 months, and (2) the Long-Term Follow-up to assess safety and pharmacodynamics for an additional four years.

1.4.1. Rationale for Dosage Selection and Method of Administration

Intravenous administration for efficient muscle transduction and the planned starting dose of 1E+14 vg/kg for the proposed clinical trial were based on the results of the animal dose-finding study (Section 1.3.2). Specifically, data from the dose finding study in the DMD rat model showed efficacy, with partial normalization of the percentage of muscle fibers expressing the human mini-dystrophin polypeptide, improvement of the tissue architecture of skeletal muscles and heart, improved muscle strength and endurance, and improvement of the cardiac diastolic function obtained at a minimal dose of 1E+14 vg/kg. In the DMD rat study (Section 1.3.2), 1E+14 vg/kg was well tolerated.

The planned second dose level of 3E+14 vg/kg for the proposed clinical study is further supported by the animal dose-finding study, with the three-fold increase in dose being associated with increased transduction and dystrophin expression in muscles. This dose was determined to be the no observed adverse effect level (NOAEL) in a juvenile rat toxicology study. However, should a dose-limiting toxicity be identified at this highest dose, subsequent

subjects of this cohort may receive a slightly lower dose of PF-06939926 at 2E+14 vg/kg or lower depending on titer method used to assess drug concentration.

The assay originally used to determine the drug concentration of PF-06939926 was based on the detection of the inverted terminal repeats (ITR) within the vector DNA sequence. In response to regulatory request, a more precise transgene (TG)-based method for assessing drug concentration has been developed. For the PF-06939926 lots used in the toxicology studies used for dose selection, the drug concentration measurements were consistent between the ITR- and TG-based titer methods. However, for the clinical investigational product (ie good manufacturing practices [GMP]) lots, PF-06939926 at 3E+14 vg/kg per the ITR-based titer method is approximately equivalent to 2E+14 vg/kg per the TG-titer method. Therefore, formal switch to the TG-based titer method per regulatory request at the second dose level, ie, PF-06939926 2E+14 vg/kg, would be in name only, and not actually a de-escalation in dose.

1.4.2. Rationale for Collection of Muscle Biopsies

This clinical trial includes up to three muscle biopsy procedures from study subjects: potentially one at Baseline, and then up to two post-infusion biopsies to assess the distribution and amount of mini-dystrophin and the persistence of transgene expression. If less than 3 biopsies are to be collected, the number and timing of biopsy collection will be instructed by Pfizer to site staff in writing. Muscle biopsies will be assessed for dystrophin expression by Western blot and/or liquid chromatography-mass spectrometry (LC-MS) and immunofluorescence staining (IFS) and will be critical to the demonstration of active pharmacology of the vector. These biopsies may also be assessed for transduction by quantitative polymerase chain reaction (qPCR) and messenger ribonucleic acid expression of the transgene by reverse transcription-polymerase chain reaction.

1.4.3. Rationale for Selected Patient Population

In order to evaluate the transduction efficiency of PF-06939926, human subjects must have adequate muscle for biopsy. In ambulatory DMD subjects, skeletal muscle exhibits fibrosis and regenerative lesions. At around the age of six years, the muscle tissue in the lower limbs begins to lose the ability to regenerate and the degenerative process becomes dominant. As necrosis continues, adipose tissue replaces muscle tissue, leading to the loss of ambulation.[42] Typically, a wheelchair becomes necessary in most cases between 12 to 15 years of age,[5] but can occur as late as 16 years of age.[43] Thus, for the ambulatory population, the age range of 4-12 years in this study, along with adequate functional ability (ie, ability to rise from floor in ≤7 seconds and walk 10 meters unassisted) should encompass subjects who will remain ambulant for at least 12 months and should have adequate muscle for transduction and for tissue biopsy for the detection of dystrophin expression.[47]

The non-ambulatory population represents a large (approximately 50%) and growing proportion of the prevalent DMD patient population which is often underserved in clinical trials.[44,45] Non-ambulatory patients have more severe disease including scoliosis, increased risk of respiratory impairment, deterioration of cardiac function, reduced limited upper limb function, and absence of lower extremity function. The near absence of the dystrophin

protein in cardiac and skeletal muscles, including those required for respiration, leads to the morbidity and mortality that characterize this disorder. The aim of gene therapy is to restore functional dystrophin in skeletal and cardiac muscle cells by expressing mini-dystrophin from the transgene and to achieve protein levels that could translate to a meaningful clinical result, including improvement or stabilization of function. The non-ambulatory population for this study is defined by the inability to walk 10 meters unassisted with no age range other than a loss of ambulation occurring at <17 years of age, to ensure consistency with a clinical phenotype of DMD. A maximum body weight has been set at 75 kg to limit the total vector genomes administered to any subject, given the unknown safety risk of high viral loads, especially in those for whom disease is more progressed. Baseline functional criteria are included to ensure that subjects have adequate muscle for biopsy and can participate safely in this study.

1.5. Anticipated Risks and Safety Monitoring

Complete information for this compound, including new potential safety risks identified during this FIH/FIP study, may be found in the Single Reference Safety Document, which for this study will be the IB.

1.5.1. Hepatic Injury

A number of on-going or completed clinical trials with AAV-based vectors have demonstrated the relative safety of this approach to treat various diseases such as hemophilia, spinal muscular atrophy (SMA) and other genetic disorders. The most consistent treatment-emergent adverse reaction has been elevations in liver enzymes, mostly related to high (>1E+12 vg/kg) vector doses. The timing of the asymptomatic liver enzyme elevations post-gene transfer has varied, but most have been adequately treated with the addition of corticosteroids with no impact on transgene expression.[48,68] Additionally, subjects treated with AAV-based vectors typically generate an immune response to the AAV capsid and may develop a cellular response to the transgene.[40,49] In an attempt to reduce any inflammatory and immune responses to the vector, subjects in an ongoing trial are now receiving steroids for several weeks after vector delivery.[49]

CCI

To address the shortcoming of the standard paradigm for monitoring of liver health in DMD patients, glutamate dehydrogenase (GLDH) has been identified as a liver-specific biomarker of liver injury that is independent of muscle damage.[50] In these studies, GLDH has been found to be as effective as ALT in diagnosing liver injury. Thus, GLDH will be used as a sensitive biomarker of liver injury in this study and may be used to guide additional

immunomodulatory intervention, as well as to confirm potential cases meeting Hy's law criteria based on elevations of bilirubin alone (in the context of elevated AST/ALT due to muscle damage).

As with other gene therapy clinical studies, elevations in liver enzymes are not unexpected. In this study, all subjects will have received glucocorticoids for at least 6 months, with a stable daily regimen for at least 3 months prior to the Baseline visit, and will continue to receive elevated daily doses of glucocorticoids for at least 3 months post-infusion of investigational product before returning to their pre-study doses of glucocorticoids for at least the remainder of the first year post-infusion. It is anticipated that the pre-existing steroid regimen along with the 3-month modified regimen may assist in controlling potential AAV-mediated liver injury.[49] However, should liver injury be detected, the glucocorticoid doses may need to be increased further, or other immunomodulatory therapy initiated, in consultation with the assigned immunopharmacologic and/or hepatic experts.

1.5.2. Immunogenicity to AAV and transgene

Humoral and cellular immune responses against both vector and the transgene may negatively impact safety and efficacy of gene therapy approaches and therefore need to be carefully monitored.[51] Humoral responses against AAV frequently occur in the population in response to spontaneous infection, with the incidence increasing with age. In fact, a single infusion with AAV leads to an effective induction of humoral immune responses.[52] Neutralizing antibodies (NAb) against the AAV capsids have been shown to negatively impact transduction.[23] Depending on the assay used to measure NAb, the prevalence of NAb to AAV9 in the general population can range from 30-40%[53] and in DMD patients pre-existing antibodies to AAV9 ranged from 10-20% depending on the assay cut-point.[54] Cellular immune response to transgene, specifically dystrophin, has been observed in clinical studies[40] and also in non-clinical studies (Section 1.3.1). In fact, a study by Flanigan (2013)[55] showed that there are pre-existing T cell responses to dystrophin in DMD patients. The study demonstrated that increasing age correlated with an increased risk for the presence of anti-dystrophin T cell responses and that treatment with corticosteroid decreased that risk compared to no treatment, suggesting that steroid treatment may modulate T cell responses.

For the reasons described above, no subjects with pre-existing NAb to AAV9 or pre-existing T cell responses to the transgene product as measured by Enzyme-Linked ImmunoSpot [ELISpot] above a certain threshold, as determined by the testing laboratories and sponsor, will initially be enrolled into this Phase 1b study. CCI

CCI

CCI In addition, systemically delivered AAV-based gene therapy is currently limited to single dose administration due to the development of NAb. Thus, subjects participating in this Phase 1b study will likely be excluded from participating in future AAV-based studies or from receiving future re-administration of PF-06939926.

Although it has not been described, either in previous dystrophin gene therapy clinical trials, or in pre-clinical models, a cellular immune response to newly and widely expressed dystrophin could theoretically induce local tissue damage related to the site of new protein expression, which would have the potential for clinical manifestation as a myositis or myocarditis. Both will be difficult to monitor in the setting of DMD, given the degree of ongoing muscle and myocardial damage related to the disease but will require a low threshold for diagnostic consideration.

A diagnosis of myositis might be considered in the event of evidence of increasing muscle pain or weakness associated with a rising creatine kinase (CK) or C-reactive protein (CRP). MRI and comparison to baseline scans may aid in the diagnosis. Myocarditis may be considered in the event of increased or new cardiac symptoms, an increased cardiac troponin I (cTnI) and creatine kinase myocardial b fraction (CK-MB), CRP, or as evidenced on electrocardiogram (ECG) or cardiac MRI (or echocardiogram).

Furthermore, the study will utilize an external data monitoring committee (E-DMC) to provide an objective assessment of safety, and which may include an expert in clinical immunopharmacology (eg, clinical immunologist, rheumatologist, transplant physician) for *ad hoc* consultation should the need arise (Section 9.6).

1.5.3. Other Risks

Possible risks related to the administration of the study drug and/or as a consequence of phlebotomy may include hematoma or bruising. If a temporary peripherally inserted central catheter (PICC) is used to facilitate the collection of blood samples, possible risks may include infection at the site, systemic infection, blood clots, bleeding, and nerve injury.

For the sirolimus cohort: possible risks associated with administration of sirolimus in a pediatric population may include risks listed in the prescribing information[71] as well as additional risks identified by review of postmarketing pediatric off label use, provided in Appendix 1.

1.5.4. Other Safety Monitoring

Additional safety monitoring is described in Section 7.2.

1.6. Other

Study C3391001 is the first gene transfer study with PF-06939926 being conducted in subjects with DMD. The drug may demonstrate pharmacologic activity in subjects. PF-06939926 is a viral vector that may transfer mini-dystrophin into muscle (and other) tissues. The gene may persist in the body for a long time; therefore, subjects will be followed for a total of 5 years post-infusion of PF-06939926.

Data from non-clinical animal testing support an acceptable risk profile for PF-06939926 in the current study and support a favorable benefit-risk profile in the indication of DMD. Authorities will be kept informed of any additional data (eg, results from clinical studies) which may affect the assessment of the benefit-risk profile for PF-06939926.

CCI [redacted]

2. STUDY OBJECTIVES AND ENDPOINTS

Primary Objective:	Primary Endpoints:
• To determine the safety and tolerability of a single IV infusion of PF-06939926 in subjects with DMD at ascending doses.	• Incidence of both dose-limiting and treatment-related adverse events (AEs), inclusive of infusion and injection site reactions, through 1 year post-treatment. • Incidence, severity, and causal relationship of treatment-emergent AEs (TEAEs) through 1 year post-treatment. • Incidence and magnitude of abnormal laboratory findings through 1 year post-treatment. • Abnormal and clinically relevant changes in physical and neurological examinations, weight, vital signs, electrocardiogram (ECG), cardiac magnetic resonance imaging (MRI)- (or echocardiogram-) measured left ventricular ejection fraction (LVEF), Columbia Suicide Severity Rating Scale (C-SSRS) through 1 year post-treatment.
• **Secondary Objectives:**	• **Secondary Endpoints:**
• To determine the amount and distribution of mini-dystrophin expression in the muscle of subjects with DMD following a single IV infusion of PF-06939926 at ascending doses. • To evaluate long term safety of a single IV infusion of PF-06939926 in subjects with DMD at ascending doses.	• Evidence of mini-dystrophin expression and transduction in muscle by immunohistochemistry (IHC) and Western Blot (WB) and/or LC-MS using muscle biopsies at 2 or 3 and 6 or 12 months post-treatment. • Incidence, severity and causal relationship of TEAEs and clinically significant safety findings, as described for the

	primary endpoints (above) through 5 years post-treatment.
CCI	

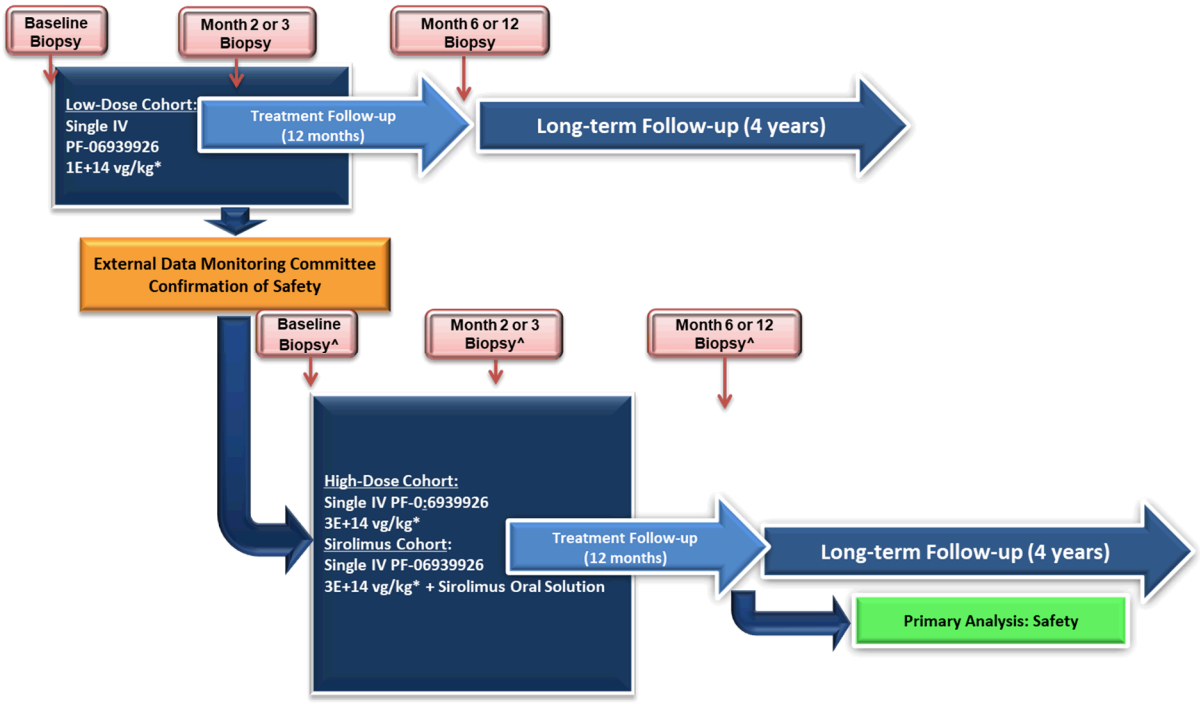
3. STUDY DESIGN

3.1. Study Overview

This study is a multi-center, open–label, non-randomized, ascending dose, safety and tolerability study of a single IV infusion of PF-06939926. There will be up to 22 subjects who will receive a single dose of PF-06939926 between 1E+14 vg/kg and 3E+14 vg/kg per the ITR-based method or 2E+14 vg/kg per the TG-titer method, as described in Section 1.4.1, with the option of de-escalating in the case of a suspected dose-limiting toxicity at this highest dose level. Up to 19 subjects who receive PF-06939926 are expected to be ambulatory upon study entry, and the remaining subjects are expected to be non-ambulatory, depending on availability of investigational product. The differences between these DMD sub-populations relate to age, weight, functional performance, and cardiac function at Screening, as described in Section 4.1 and Section 4.2. Sites will be kept updated of study enrollment status to mitigate risk of over-enrollment of either sub-population.

In order to mitigate unanticipated risks to subject safety, enrollment will be staggered within and between the two cohorts and will include a formal review by the E-DMC prior to dose progression and in the event of any possible safety signals (see Section 3.5). Any and all changes to dose-level will be communicated to sites by formal letter, regardless of reason (eg, changes in the name only due to development of a more precise titer assay, escalation or de-escalation following E-DMC reviews, etc.). Within each of the cohorts, the dosing interval between the first and second subjects will be at least 6 weeks. If no safety concerns are identified 3 weeks after the second subject is infused, then dosing may proceed at ⩾ 2-3-week intervals. Similarly, if no stopping or pause criteria (as described in Section 3.5) are met after dosing 6 consecutive subjects at a single dose level, then, with E-DMC agreement, dosing may proceed at that same dose level in ≥1-week intervals. A visual description of the study design is provided in Figure 2.

Figure 2. Study Schematic



*Dose-level determined by inverted terminal repeat (ITR)-titer based assay, but PF-06939926 3E+14 vg/kg using the ITR- based titer assay is approximately equivalent to PF-06939926 2E+14 vg/kg under the transgene (TG)-based titer assay.
^Number of biopsies may be reduced or eliminated for the non-ambulatory subjects as per sponsor discretion.

3.2. Planned Number of Subjects

Depending on availability of investigational product, up to 22 subjects, including up to 19 ambulatory subjects and 3 non-ambulatory subjects, including those participating in the sirolimus cohort, are planned to participate in up to 5 centers in the United States (US).

Subjects who are withdrawn for reasons other than safety may be replaced at the discretion of the sponsor.

It is anticipated that approximately 40% of the general population may have detectable NAb to AAV9[53] which would preclude participation in this trial (see Exclusion Criteria). Thus, in consideration of this criterion and the other eligibility criteria, approximately 37 subjects may need to be screened in order to meet the overall enrollment target. In light of this screen failure rate, efforts will be made to minimize subject burden by obtaining the NAb results prior to conducting the other Screening procedures, when feasible, while also balancing the timing of the Screening visits with the staggered enrollment. Such efforts will require close communication between the site staff and sponsor study team.

3.3. Duration of Subject Participation

Willing and eligible subjects will participate in the trial for approximately 5.5 years, and visits will include the following:

- Screening: approximately 2 days of assessments across 2-3 visits within an approximately 26-week period relative to the Baseline visit;
- Baseline: approximately 2 days of assessments across 1-2 visits within a 2-week period;
- Treatment: approximately 2 visits: 1) single administration of PF-06939926 followed by at least 24 hours of in-patient monitoring (ie Days 1 and 2), and 2) 2-week follow-up in which subjects will remain local to the investigational site, and complete 1-day visits occurring on Days 4, 7, and 10 (±1 day) for blood sample collection and safety monitoring, as well as sample collection for local laboratory testing on Days 5-9, or longer, as clinically indicated;
- 1st Year Follow-up: approximately 1 to 3 days of assessments for 6 visits, as well as 8 remote visits (eg, blood sample collection at subject's home, virtual wellness check-ins that include AE assessments, activity monitoring), during the first 12 months following the single administration of PF-06939926;
- Long-Term Follow-up: total of 4 annual, 2 or 3-day visits starting approximately 2 years following the single administration of PF-06939936, as well as 4 remote visits (eg, blood sample collection in the subject's home, virtual wellness check-ins) in between the 4 annual visits.

3.4. Approximate Duration of Study

The entire study is estimated to complete in approximately 8 years, allowing for approximately 3 years of staggered enrollment and 5 years of follow-up.

The end of the study will be the last visit of the last subject for purposes of closing out sites, informing the institutional review board/ethics committee (IRB/EC), and ceasing to send Council for International Organizations of Medical Sciences (CIOMS) reports.

3.5. Dose Progression and Stopping Rules

As described above (Section 3.1), 2 cohorts are planned in this study. The available results of the 2-month muscle biopsies of the enrolled subjects will be reviewed prior to dosing the 4th subject in the low-dose cohort, and in either of the following cases, an *ad hoc* E-DMC review of the available cumulative safety data may be requested to assess whether escalation to the high-dose cohort may commence:

1. No measurable transgene (mini-dystrophin) noted in 2 of the first 3 enrolled subjects, or

2. Level of transgene expression is regarded by the sponsor as inadequate to translate into functional benefit.

If adequate transgene expression is observed, dosing of the remaining subjects in the low-dose cohort will continue as planned, after which an E-DMC review of available cumulative safety data will occur.

In any circumstance, progression to the high-dose cohort will not proceed until the first dose level is deemed safe by the E-DMC, principal investigators and sponsor study team.

If no transgene (mini-dystrophin) expression is observed in any of the 2-month biopsies from the low-dose cohort, then prior to dosing the 4th subject in the high-dose cohort, all available biopsies may be assessed. If these samples are also negative for transgene (mini-dystrophin) expression, an *ad hoc* E-DMC review of the available cumulative safety data may be requested to determine whether the study should be terminated.

For both cohorts and at any dose level of PF-06939926, if one of the following safety issues occurs, further enrollment will be halted until the E-DMC reviews data for the subjects with the clinical events that prompted the review and makes recommendations as to next steps:

- Any potentially treatment-related serious adverse event (SAE);
- Similar clinically significant safety findings in ⩾50% of subjects at a given dose level, indicating dose-limiting intolerance;
- Repeated alkaline phosphatase or total bilirubin >2 × the upper limit of normal (ULN);
- Any set of laboratory results meeting Hy's Law (see Section 8.4.2 for Hy's Law criteria);
- Clinical diagnosis of myositis or myocarditis;
- Other findings that, at the discretion of the sponsor study team, investigator and/or E-DMC, indicate that dose escalation should be halted or that dose de-escalation would be appropriate.

Additional information on the E-DMC can be found in Section 9.6.

4. SUBJECT ELIGIBILITY CRITERIA

This study can fulfill its objectives only if appropriate subjects are enrolled. The following eligibility criteria are designed to select subjects for whom participation in the study is considered appropriate. All relevant medical and nonmedical conditions should be taken into consideration when deciding whether a particular subject is suitable for this protocol.

Subject eligibility should be reviewed and documented by an appropriate member of the investigator's study team before subjects are included in the study.

4.1. Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for enrollment into the study:

1. Evidence of a personally signed and dated informed consent and/or assent (where appropriate) document indicating that the subject or a legally acceptable representative/parent(s)/legal guardian has been informed of all pertinent aspects of the study (see Section 12.3 for additional information regarding the consenting process).

2. Willingness and ability by subject, as well as parent(s)/legal guardian(s) when appropriate, to comply with scheduled visits, treatment plan, laboratory tests, and all other required study procedures.

3. Males, who are aged as follows based on Ambulatory Status (ie, ability to walk 10 meters unassisted):

   - *FOR AMBULATORY SUBJECTS*: 4 to 12 years, inclusive;

   - *FOR NON-AMBULATORY SUBJECTS:* No age restrictions so long as loss of ambulation occurs prior to the subject's 17th birthday.

4. Body weight, as follows:

   - *FOR AMBULATORY SUBJECTS: ⩾15 to ⩽50 kg;*

   - *FOR NON-AMBULATORY SUBJECTS:* Up to 75 kg, but enrollment will be controlled by steady increases in body weight to enable cautious accumulation of safety information across the broad weight range. Unless the sponsor formally informs the site staff otherwise, enrollment will occur such that a minimum of 2 subjects in each of the following weight ranges will be dosed in the following order:

     - >35 to ⩽50 kg;

     - >50 to ⩽60 kg;

     - >60 to ⩽75 kg.

5. Diagnosis of Duchenne muscular dystrophy (DMD) confirmed by the subject's medical history and by genetic testing prior to screening. Note that the latter may require confirmation by repeat testing by an appropriately regulated, central laboratory.

6. Receipt of glucocorticoids for ⩾6 months and a stable daily dose (⩾0.25 mg/kg/day prednisone, prednisolone, or deflazacort) for at least 3 months prior to the Baseline visit.

7. Functional performance as follows, based on Ambulatory Status (ie, ability to walk 10 meters unassisted):

   - *FOR AMBULATORY SUBJECTS*, defined as those able to walk 10 meters unassisted as part of Ambulatory Status, AND with

     - Ability to rise from floor within seven (7) seconds (as part of NSAA);

   - *FOR NON-AMBULATORY SUBJECTS*, defined as those unable to walk 10 meters unassisted, AND with:

     - Adequate respiratory function as evidenced by percent predicted forced vital capacity (%pFVC) >40% (as part of pulmonary function), AND

     - Adequate upper limb function as evidence by the PUL, version 2.0, Entry Item A score of ⩾3.

NOTE: Although a window of up to 26 weeks (182 days) between Screening and Baseline is permitted, if time between Screening and the planned Baseline visit exceeds 12 weeks (84 days), then these functional assessments should be repeated and eligibility (re)confirmed prior to enrolling the subject.

8. Ability to tolerate magnetic resonance imaging (MRI) without sedation and with no contraindications to these procedures.

9. Ability to tolerate muscle biopsies under anesthesia with no contraindications to these procedures.

4.2. Exclusion Criteria

Subjects with any of the following characteristics/conditions will not be included in the study (ie, will not receive PF-06939926):

1. Receipt of a live attenuated vaccination within 3 months prior to receipt of PF-06939926 or exposure to an influenza (or other inactivated) vaccination or systemic antiviral and/or interferon therapy within 30 days prior to receipt of PF-06939926.

2. Current exposure to systemic immunosuppressant agents other than glucocorticoids.

3. The following genetic abnormalities in the dystrophin gene as confirmed by the investigator based on the review of the DMD genetic testing:

    a. Any mutation (exon deletion, exon duplication, insertion, or point mutation) affecting any exon between exon 9 and exon 13, inclusive; OR

    b. A deletion that affects both exon 29 and exon 30.

4. Prior treatment with gene therapy, defined as any therapy introducing exogenous DNA or intended to permanently alter the endogenous DNA. Gene therapy (other than study drug) will be prohibited for the duration of the study.

5. Exposure within 6 months prior to Screening (Visit 1) to any treatment designed to increase dystrophin expression (including, but not limited to exon-skipping agents and nonsense read-through). These treatments will also be prohibited during the period between Screening (Visit 1) and Day 1 (Visit 3) and for the first year of the study.

6. Participation in other studies involving investigational drug(s) within 30 days or within 5 half-lives, whichever is longer, prior to the Baseline visit.

7. Known hypersensitivity to any of the components of the study drug or solution for infusion, such as hypersensitivity to albumin or a diagnosis of (or symptoms suggestive of) hereditary fructose intolerance (HFI). Symptoms suggestive of HFI include nausea, vomiting, bloating, stomach cramps, or diarrhea following the ingestion of sweet foods or drinks, or a pattern of avoiding sweet foods or drinks.

8. NAb against AAV9 above threshold set by testing laboratory at Screening ***(and Baseline if required See*** Schedule of Activities***, footnote i)***.

9. Abnormality in hematology or chemistry profiles at screening:

    a. Absolute neutrophil count (ANC) <1000 cells/mm^3;

    b. Cystatin C >1.2 x ULN;

    c. Platelets <150 × $10^3/\mu L$;

    d. Positive hepatitis A virus (anti-HAV) immunoglobulin M (IgM), hepatitis B surface antigen (HBsAg), and/or hepatitis C antibody (HCVAb);

    e. Serum markers indicating possible autoimmune-mediated hepatitis:

        1. Antinuclear antibody (ANA) titer >1:160;

        2. Total IgG >2 x ULN;

f. Markers of hepatic inflammation or overt or occult cirrhosis as evidenced by one or more of the following:

i. Total bilirubin >1.5 x ULN and direct bilirubin ⩾0.5 mg/dL;

3.GGT >1.5 x ULN;

4.PT > ULN; prolonged INR >ULN;

5.GLDH >2 x ULN;

NOTE: Although a window of up to 26 weeks (182 days) between Screening and Baseline is permitted, if time between Screening labs and the planned Baseline visit is to exceed 12 weeks (84 days), then all Screening labs except for DMD genetic testing should be repeated and eligibility (re)confirmed prior to enrolling the subject.

10. Compromised cardiac function as indicated by a left ventricular ejection fraction (LVEF) on cardiac MRI obtained during or within 3 months prior to Screening, as evaluated by a central imaging vendor, and based on ambulatory status as follows:

- *FOR AMBULATORY SUBJECTS*: LVEF of <55%;
- *FOR NON-AMBULATORY SUBJECTS*: LVEF of <35%.

NOTE: If the cardiac MRI is unable to be read (eg, due to an inability by a younger subject to remain still during the scan), then echocardiogram may be performed and read locally.

11. Any injury which may impact functional testing. Previous injuries must be fully healed prior to consenting. For ambulatory subjects, prior lower limb fractures must be fully healed and at least 3 months from injury date.

12. Clinically significant infection within 30 days prior to study drug administration, as determined by the investigator.

13. Refusal or inability to use acceptable forms of birth control for the duration of the study and through completion of final study visit (Section 4.5.2).

14. Presence or history of other musculoskeletal or neurological disease in addition to DMD.

15. Evidence or history of clinically significant hematological, renal, endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, cancer, autoimmune or allergic disease (including drug allergies), but excluding untreated, asymptomatic, seasonal allergies at time of dosing.

16. Other acute or chronic medical or psychiatric condition including recent (within the past year) or active suicidal ideation or behavior (within the past 6 months) or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the subject inappropriate for entry into this study.

17. Investigator site staff members directly involved in the conduct of the study and their family members, site staff members otherwise supervised by the investigator, or subjects who are Pfizer employees, including their family members, directly involved in the conduct of the study.

NOTE: Screening results considered by the investigator to be transient and inconsistent with the subject's clinical condition may be repeated once during the Screening period for confirmation of eligibility. The reason for repeating the assessment should be documented.

4.3. Caregiver(s)

The parent(s) or legal guardian(s) of the subject will actively participate as caregiver in this study. As caregiver(s), the parent(s) or legal guardian(s) will not only provide informed consent for those subjects who are <18 years of age (or as appropriate, per Section 12.3), but will also actively participate in the study at least for subjects who are minors, including attendance at study visits, exchanges during wellness check-ins, and completion of patient-reported questionnaires on behalf of the subject as well as himself and herself. With consent, the caregiver's demographic information may be collected. The caregiver(s) will also communicate observed safety information to the investigator or designee as appropriate.

For subjects who are minors, a caregiver(s) must meet all of the following criteria for the subject to be eligible for enrollment in the study:

- Aged ⩾18 years of age and has demonstrated responsibility as a legal caregiver(s) through monitoring the subject and reporting any observed AEs;
- Willingness and ability to provide written informed consent on behalf of the subject;
- Ability to accompany the subject to the clinic visits and complete certain assessments on behalf of the subject, as well as for themselves, if willing;
- Ability to follow instructions.

4.4. Re-screening

Subjects who are screened but ultimately discontinued prior to receipt of PF-06939926 for reasons other than a confirmed, non-transient failure to meet all eligibility criteria (eg, enrollment pause due to regulatory or safety review, operational issues causing significant delays, etc) may be allowed to re-screen during the study while enrollment remains open. In this case, the subject number assigned at the initial screening will be documented as a screen failure the subject would be issued a new subject number for the

re-screen. Re-screened subjects will re-consent, and screening assessments will need to be repeated, as described in Section 6.1.

4.5. Lifestyle Requirements

4.5.1. Activity

Subjects should be instructed to continue with routine physical therapy including stretching or use of orthoses to prevent or minimize contractures or muscle deformities.

Subjects will be instructed to maintain normal activity levels and avoid activities that are not part of their normal daily routine within 24 hours of study visits where imaging or functional assessments will be performed.

As this study involves a viral vector, it is suggested that subjects and those around them be reminded to practice good general hygiene for the first 1-2 months after receiving PF-06939926, such as the following: (1) thoroughly washing hands with soap after using the bathroom, before preparing or touching food, or after blowing one's nose, coughing, sneezing, etc.; (2) avoiding contact with persons who might be sick; and (3) not sharing glasses, dishes, or utensils with other individuals.

In addition, for the first month following administration of PF-06939926, it is suggested that the caregivers assist the subjects with monitoring fluid intake and output. Specifically, the recommendations are that subjects consume at least 1 liter of water per day and pass urine at least 3 times during each 24-hour period through the first month, or longer if deemed clinically necessary. If fluid intake and output are observed to be less than these thresholds, then subjects (and/or caregivers) should be instructed to promptly contact the site for a possible unscheduled visit and/or biospecimen collection.

4.5.2. Contraception

All subjects who are, in the opinion of the investigator, able to father children and sexually active and at risk for pregnancy with their partner(s) must agree to use a highly effective method of contraception consistently and correctly for the duration of the study, including long-term follow-up. The investigator or his or her designee, in consultation with the subject and/or the subject's legal caregiver (depending on the subject's age and appropriateness of this sensitive topic), will confirm that the subject has selected an appropriate method of contraception for the individual and partner from the permitted list of contraception methods (see below) and will confirm that the subject has been instructed in its consistent and correct use. Throughout the study, the investigator or designee will inform the subject and/or legal caregiver of the need to use highly effective contraception consistently and correctly and document the conversation and the subject's affirmation in the subject's chart (subjects need to affirm their consistent and correct use of at least 1 of the selected methods of contraception). In addition, the investigator or designee will instruct the subject and/or legal caregiver to call immediately if the selected contraception method is discontinued or if pregnancy is known or suspected.

Highly effective methods of contraception are those that, alone or in combination, result in a failure rate of less than 1% per year when used consistently and correctly (ie, perfect use) and include the following:

1. Male condom used WITH a separate spermicide product (ie, foam, gel, film, cream, or suppository);

2. Male sterilization with absence of sperm in the post-vasectomy ejaculate.

NOTE: Sexual abstinence, defined as completely and persistently refraining from all heterosexual intercourse (including during the entire period of risk associated with the study treatments) may obviate the need for contraception ONLY if this is the preferred and usual lifestyle of the subject.

4.6. Sponsor's Qualified Medical Personnel

The contact information for the sponsor's appropriately qualified medical personnel for the study is documented in the study contact list located in the supporting sponsor study team electronic storage site and/or study portal.

To facilitate access to appropriately qualified medical personnel on study-related medical questions or problems, subjects are provided with a contact card. The contact card contains, at a minimum, protocol and investigational product identifiers, subject study numbers, contact information for the investigator site, and contact details for a contact center in the event that the investigator site staff cannot be reached to provide advice on a medical question or problem originating from another healthcare professional not involved in the subject's participation in the study. The contact number can also be used by investigator staff if they are seeking advice on medical questions or problems; however, it should be used only in the event that the established communication pathways between the investigator site and the sponsor study team are not available. It is therefore intended to augment, but not replace, the established communication pathways between the investigator site and the sponsor study team for advice on medical questions or problems that may arise during the study. The contact number is not intended for use by the subject directly, and if a subject calls that number, he or she will be directed back to the investigator site.

5. STUDY TREATMENTS

For the purposes of this study, and per International Conference on Harmonisation (ICH) guidelines, investigational product is defined as a pharmaceutical form of an active ingredient or placebo being tested or used as a reference/comparator in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use (ICH E6 1.33).

For this study, the investigational product is PF-06939926.

Refer to the Investigational Product Manual and the Administration Card for details regarding the preparation and administration of this investigational product.

Subjects will be treated with a single IV infusion of PF-06939926. Please refer to Investigational Product Manual for complete information on storage, stability, preparation and administration of PF-06939926.

This study is an open-label, non-randomized study in which subjects will receive a single IV infusion of an ascending dose of PF-06939926. Subjects in the low-dose cohort will receive a single infusion containing 1E+14 vg/kg, and subjects in the high-dose cohort are planned to receive a single infusion containing 3E+14 vg/kg per ITR-based titer method. However, should a dose-limiting safety event occur at this highest dose level, subsequent subjects may be administered 2E+14 vg/kg or lower per ITR-based titer method. The dose will be calculated based on the subject weight, which will be obtained at the Baseline visit (see Section 5.3.1).

To mitigate risk of an immune response and drug-induced liver injury, as described in Section 1.5, subjects will temporarily replace their daily glucocorticoid regimen as follows:

1. Between 1 and 4 hours prior to infusion of PF-06939926: receipt of single dose of IV methylprednisolone ⩾2 mg/kg;

2. For first 2 weeks post-infusion of PF-06939926: switch of daily glucocorticoid dose to ⩾2 mg/kg/day of oral prednisone or prednisolone;

3. For Weeks 3 and 4 post-infusion of PF-06939926: reduction of daily glucocorticoid dose to ⩾1.5 mg/kg/day of oral prednisone or prednisolone;

4. For Weeks 5, 6, 7, and 8 post-infusion: reduction of oral prednisone or prednisolone dose to a minimum of ≥1.0 mg/kg/day;

5. For Weeks 9, 10, 11, and 12 post-infusion: reduction of oral prednisone or prednisolone dose to a minimum of 0.75 mg/kg/day;

6. After Week 12 post-infusion: reduction of oral prednisone or prednisolone dose to a minimum of 0.5 mg/kg/day or return to pre-study daily doses of prednisone or prednisolone, whichever dose is higher.

These doses will be calculated within approximately 10% of the subject's body weight. Provided that no signals of a latent immune response are present following these 3 months of descending dose levels of prednisone or prednisolone, subjects may then return to their pre-study dose levels of daily glucocorticoids (eg, prednisone, prednisolone, or deflazacort) for at least the remainder of the first year following receipt of PF-06939926.

5.1. Allocation to Treatment

The investigator's knowledge of the treatment should not influence the decision to enroll a particular subject or affect the order in which subjects are enrolled.

Allocation of subjects for treatment will be performed by the sponsor's study management team. An interactive response technology (IRT) system (interactive Web-based response [IRT] will be used for subject screening and enrollment. The site personnel (study coordinator or specified designee) will be required to enter or select information including but not limited to the user's identification (ID) and password, the protocol number, and the subject-specific identification number (SSID), the subject's weight collected at Baseline (Section 5.3.1) and subject's date of birth. The site personnel will then be provided with an enrollment number via the IRT system. The IRT system will provide a confirmation report containing the subject number, and enrollment number. The confirmation report must be retained by the dispenser in the site files.

Subjects will be allocated to the open dose cohort according to their enrollment number. Once subject screening and enrollment numbers have been assigned, they cannot be reassigned.

The study-specific IRT reference manual will provide the contact information and further details on the use of the IRT system.

5.2. Subject Compliance

Investigational product will be administered by the appropriately designated study staff at the investigator site.

5.3. Investigational Product Supplies

5.3.1. Dosage Form(s) and Packaging

The amount of study drug shipped to site will be based on subject's Baseline body weight and the lot-specific concentration of PF-06939926. A shipment order will be raised once the subject is registered (ie, enrolled) following confirmation of eligibility. Thus, it is highly recommended that all subject-level details, especially body weight, are confirmed by two (2) site personnel prior to enrolling the subject, so as to ensure that the appropriate amount of study drug is sent to the site for dosing. Guidance for collecting the most accurate weight is provided in Section 7.2.4.2. As a minimum of 2 weeks (14 calendar days) is required for shipment of study drug, enrollment should occur promptly following the measurement of the subject's weight at Baseline.

The dose will be prepared by the site pharmacist following the instructions in the Investigational Product Manual.

PF-06939926 will be supplied as a sterile, frozen solution requiring thawing and further dilution for IV administration. The drug product is supplied in a clear closed vial configuration with stopper and a cap in place. Each vial contains a 5 mL nominal fill volume of PF-06939926. Each lot of PF-06939926 will have a lot-specific titer concentration.

Details of the drug product packaging, accompanying shipment documentation and drug product preparation are provided in the Investigational Product Manual.

All ancillary supplies used to prepare and administer doses will be provided by the clinical site conducting the study unless otherwise agreed by the sponsor.

5.3.2. Preparation and Dispensing

See the Investigational Product Manual for instructions on how to prepare the investigational product for administration. Investigational product should be prepared and administered by appropriately qualified and experienced members of the study staff (eg, physician, nurse and pharmacist as allowed by local, state, and institutional guidance). The subject's weight from Baseline will be used for calculating the dose. All dosage calculations, as well as dose preparation, must be performed and checked by a minimum of two (2) qualified clinical site personnel.

PF-06939926 vials are single use only.

Only qualified personnel who are familiar with procedures that minimize undue exposure to themselves and to the environment should undertake the preparation, handling, and safe disposal of gene therapy, specifically AAV-vector based agents. Additional details regarding inadvertent exposure and instructions on spill cleanup procedures are outlined in the "Investigational Product Manual".

5.4. Investigational Product Administration

Delivery of premedication with IV methylprednisolone ⩾2 mg/kg between 1 and 4 hours prior to infusion is required for all subjects in lieu of daily oral glucocorticoid.

Subjects will be admitted to the investigational site to receive a single intravenous infusion of PF-06939926 delivered over approximately 2 to 4 hours (+30 minutes) at Visit 3 in accordance with the Schedule of Activities and will be monitored for at least 24 hours following completion of the infusion.

The appropriate amount of PF-06939926, based on the actual concentration for the lot, the subject's Baseline body weight and the dose level, will be prepared and administered in a solution of approximately 250 mL to 500 mL containing a small amount of human serum albumin as detailed in the Investigational Product Manual. Appropriate medication and other supportive measures for management of an acute hypersensitivity reaction should be available in accordance with local guidelines for standard gene therapy practices.

Preparation and administration of investigational products should be performed by appropriately qualified, Good Clinical Practice (GCP)-trained, and preferably gene therapy-experienced members of the study staff (eg, physician, nurse, physician's assistant, nurse practitioner, pharmacist, or medical assistant) as allowed by local, state, and institutional guidance.

Should subjects experience any infusion site reaction during the IV infusion period, the administration should be interrupted, and supportive care should be provided according to the investigator's standard of care practice (eg, treatment with an antihistamine and/or glucocorticoid, etc). The administration can be restarted based on the medical judgement of

the investigator. It is recommended that the investigator assess the infusion reaction and establish new IV access if needed, as every reasonable effort should be made to complete the entirety of the dose. The study drug solution has 8 hour stability at room temperature from the start of dose preparation (initial study drug vial puncture). If there is an interruption to the infusion, the 8 hour in-use period cannot be extended, and every effort should be made to resume and complete the administration within this period. The time at which the infusion was paused, the volume infused prior to pausing the infusion, and the time at which the infusion resumes should all be noted in source documentation by those involved in study drug administration in addition to documentation required at end of infusion.

Investigational product administration details will be recorded on the case report form (CRF) within the clinical trial database.

5.5. Investigational Product Storage

The investigator or an approved representative, eg, pharmacist, will ensure that all investigational products are stored in a secured area with controlled access under required storage conditions and in accordance with applicable regulatory requirements for gene therapy.

Investigational products should be stored in their original containers and in accordance with the labels.

PF-06939926 sterile, cryogenic vials must be stored frozen at -60 to -90°C.

The Investigational Product Manual will contain instructions on thawing the sterile, cryogenic vials and for storage conditions of the product once thawed and/or diluted for administration.

Any storage conditions stated in the SRSD (eg, IB) will be superseded by the storage conditions stated on the product label.

Site systems must be capable of measuring and documenting (for example, via a log), at a minimum, daily minimum and maximum temperatures for all site storage locations (as applicable, including frozen, refrigerated, and/or room-temperature products). This should be captured from the time of investigational product receipt throughout the study. Even for continuous-monitoring systems, a log or site procedure that ensures active evaluation for excursions should be available. The intent is to ensure that the minimum and maximum temperature is checked each business day to confirm that no excursion occurred since the last evaluation and to provide the site with the capability to store or view the minimum/maximum temperature for all non-working days upon return to normal operations. The operation of the temperature monitoring device and storage unit (ie, freezer) should be regularly inspected to ensure they are maintained in working order.

Any excursions from the product label storage conditions should be reported to Pfizer upon discovery. The site should actively pursue options for returning the product to the storage

conditions described in the labeling, as soon as possible. Deviations from the storage requirements, including any actions taken, must be documented and reported to Pfizer.

Once an excursion is identified, the investigational product must be quarantined and not used until Pfizer provides permission to use the investigational product. It will not be considered a protocol deviation if Pfizer approves the use of the investigational product after the temperature excursion. Use of the investigational product prior to Pfizer approval will be considered a protocol deviation. Specific details regarding information the site should report for each excursion will be provided to the site.

5.6. Investigational Product Accountability

The investigator site must maintain adequate records documenting the receipt, use, loss, or other disposition of the investigational product supplies. All investigational products will be accounted for using a drug accountability form/record.

5.6.1. Destruction of Investigational Product Supplies

The sponsor or designee will provide guidance on the destruction of unused investigational product (eg, at the site). The investigator must ensure that the materials are destroyed in compliance with applicable environmental regulations, institutional policy, and any special instructions provided by Pfizer, and all destruction must be adequately documented.

5.7. Concomitant Treatment(s)

- Subjects will abstain from all prohibited concomitant medications/treatments, except if required for treatment of AEs;

- All concomitant medications taken during the study will be recorded with the indication and start and stop dates of administration. All subjects will be questioned about concomitant medications at each visit, including compliance with the required daily glucocorticoid regimen through at least the first year of follow-up;

- Medications taken within 28 days prior to Day 1 will be documented as prior medication. Medications taken from the time of Day 1 will be documented as concomitant medications.

5.7.1. Permitted Therapies

- Prior to enrollment, subjects will be required to have received glucocorticoids (eg, deflazacort, prednisolone, or prednisone) for at least 6 months and with no significant changes (>0.2 mg/kg) in a daily dose regimen for at least 3 months prior to the Baseline visit. The daily dose levels of the glucocorticoids should remain the same throughout the first year of follow-up with the exception of the following:

    1. Planned temporary increases during the first few months following administration of study drug (Section 5);

    2. Increases due to a potential drug toxicities (Section 5.8);

3. Slight adjustments due to changes in weight (ie, <0.2 mg/kg).

- Subjects will also receive anesthesia according to the site standard of care for the muscle biopsies obtained in this study;

- Subjects who have no contraindications and who have not previously received a MenACWY vaccination; or whose last vaccination at the time of the Screening visit (Visit 1) is outside the time period of active coverage specified by the vaccine manufacturermust receive at least 1 dose of MenACWY vaccine as early as possible in the Screening Period and not later than 30 days prior to receiving PF-06939926 and may receive prophylactic antibiotics in the event that the eculizumab is required to treat an immune response to PF-06939926, as described in Section 5.8;

- Subjects must receive MenB vaccination if indicated by national vaccination guidelines no later than 30 days prior to receiving PF-06939926;

- Subjects will be permitted to receive ACE (angiotensin-converting enzyme) inhibitors, β blockers, ARBs (angiotensin II receptor blockers) or aldosterone blocker/thiazide diuretics;

- Supplements such as vitamin D, coenzyme Q10, carnitine, amino acids (glutamine, arginine), anti-inflammatory/anti-oxidants (eg, fish oil, vitamin E, green-tea extract) are permitted;

- Bisphosphonates are permitted;

- With the exception of any prohibited therapies (see next section), other medications may be deemed necessary by the investigator either to treat or prevent adverse events that may be related to PF-06939926. Examples of these may include but are not limited to antiemetics or antihistamines for anticipated or observed nausea and vomiting events, an increase in dose of glucocorticoids or addition of other immunosuppressants or complement inhibitors for anticipated or observed events associated with complement activation, as described in the IB.

5.7.2. Prohibited Therapies

The following are prohibited from the time of signing the informed consent through the final study visit:

- Immunosuppressant agents, (other than glucocorticoids) unless administered in response to or to mitigate risk of immunologic reaction;

- Other investigational therapies, including idebenone and tamoxifen;

- Anti-myostatin, utrophin modifiers, and other gene therapy agents;

- Antiviral therapy, unless administered to treat an acute viral infection (eg, COVID-19);
- Interferon therapy, unless administered to treat an acute viral infection and used at least 2 months after administration of PF-06939926;
- Sedation for imaging assessments.

The following are prohibited during the period between Screening (Visit 1) and Day 1 (Visit 3) and for the first 3 months following administration of PF-06939926:

- Receipt of any required live attenuated vaccine(s);
- Receipt of an influenza (or other inactivated) vaccine(s);
- Receipt of an mRNA, or DNA-based, or non-replicating viral vector vaccine (eg, against SARS-CoV2).

The following are prohibited during the period between Screening (Visit 1) and Day1 (Visit 3) and for the first year of the study:

- Any treatment designed to increase dystrophin expression, (including, but not limited to exon-skipping agents and non-sense read through).

5.8. Rescue Medication

Should subjects experience an infusion site reaction during the IV infusion period, the treatment administration should be paused for the subject and supportive care should be provided according to the investigator's standard practice (eg, treatment with an antihistamine).

It is anticipated that the pre-study steroid regimen along with the 3-month post-dose glucocorticoid regimen (Section 5) may assist in controlling potential AAV-mediated liver injury in humans.[49] However, should liver enzymes (ie, GLDH and bilirubin, Section 1.5.1) become elevated during the study, the glucocorticoid doses may need to be further increased or other immunomodulatory treatment added in *ad hoc* consultation with the assigned immunopharmacologic and/or hepatic experts.

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CCI However, because the use of eculizumab increases the risk of contracting serious meningococcal infections, it is required that subjects not previously vaccinated receive at least 1 dose of meningococcal vaccination(s) at least 30 days prior to administration of PF-06939926 (ie, Baseline visit). Alternatively (eg, in the case of allergy),

prophylactic antibiotics for meningococcus should be provided simultaneously with eculizumab, if administered. In addition, the planned glucocorticoid doses may be increased and/or other immunosuppressants may be administered to treat or prevent an adverse immune reaction, such as those described above.

Subjects who experience an event compatible with aHUS may be screened for a genetic predisposition to aHUS, as determined by the investigator in consultation with the sponsor to increase the understanding of the risk of developing aHUS (see Table 1).

6. STUDY PROCEDURES BY VISIT

Every attempt should be made to schedule the visits on the day specified in the Schedule of Activities. If possible, in order to provide optimal testing conditions and consistency in endpoint measurements, functional assessments and imaging would be best performed on two separate days within the visit window and at *approximately* the same time of day in the order described below.

The visit windows are described below.

6.1. Visit 1 (Screening): Days -196 to -17

This visit will be conducted over a minimum of 2 days to separate the days when the MRI of thigh and upper limb and functional assessments are conducted. Sites may choose to extend this beyond 2 days to accommodate the logistics of completing all testing. It is *highly* recommended that the blood draw for immunogenicity testing be taken at the beginning of the Screening period due to the processing time for these assays.

During Screening, subjects and caregiver(s) will be assessed for study eligibility. All Screening assessments must be completed and reviewed for subject eligibility before the subject is enrolled into the study. Screening tests with results considered by the investigator to be transient and inconsistent with the subject's clinical condition may be repeated once during the Screening period for confirmation of eligibility. Imaging-based examinations for Screening must be reviewed for quality by the central imaging vendor before the subject is enrolled to assure an adequate baseline image has been acquired. If the image is determined to be of poor quality, it may be repeated. The visit window (Day -196 to -17) for screening is to allow for the analysis of laboratory testing, assurance of imaging quality and to provide multiple days to perform the assessments in the order described below. However, certain Screening assessments, namely laboratory tests, functional assessments, thigh and upper limb MRI, would need to be repeated prior to enrollment should the time between these and the planned Baseline visit exceed 12 weeks (84 days), as described in Section 4.2.

If a subject is withdrawn from the study prior to receiving PF-06939926, but wishes to be re-screened and meets the criteria described in Section 4.4, this subject must be re-consented. In addition, all Screening assessments must be repeated with the exception of genetic testing to confirm DMD diagnosis, as well as cardiac imaging, the latter of which would only be repeated if >6 months from when first obtained.

Assessments to be made for the Screening Visit include:

- **Informed Consent/Assent:** Prior to initiation of any screening assessments the subject (if age of majority, as per Section 12.3) or subject's parent(s) or legal guardian(s) must sign the informed consent document (ICD). If a minor, the subject will be required to provide assent in compliance with local regulations and IRB/EC requirements;

- **Demographics:** Information such as date of birth, race, ethnicity, and gender will be collected;

- **Medical History:** Medical history will include confirmation by genetic testing of the diagnosis of DMD as reported from an appropriate regulated laboratory using a clinically-validated genetic test. Results must confirm the presence of a mutation in the dystrophin gene(s) which is clinically consistent with the diagnosis of DMD. The mutation type will be reported. Medical history will also be reviewed for any significant medical histories and concurrent illnesses that required or are requiring specialist consultation or treatment;

- **Medication History:** Complete history will include all prescription or nonprescription drugs, and dietary and herbal supplements taken within 28 days prior to the planned first dose. The history of glucocorticoid use for at least 6 months with 3 months on a stable, daily dose prior to the Baseline visit will be collected to confirm eligibility;

- **Inclusion/Exclusion Criteria:** Subjects will be assessed against inclusion and exclusion criteria;

- **Physical Examination and Neurological Examination;**

- **Height and Weight** for determination of eligibility (ie, weight) and should be measured in morning, when feasible;

- **Vital Signs:** Blood pressure, pulse rate, and respiratory rate, and, if clinically indicated, body temperature;

- **Triplicate 12-lead ECG;**

- **Clinical laboratory assessments:**

    - *Blood samples*:

    - Confirmation of genetic testing of the diagnosis of DMD to be collected *as early as possible in the screening period* after informed consent;

    - [redacted]

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- Clinical safety, including hematology, chemistry and other labs as described in Table 1.

- Anti-HAV IgM, HBsAg, HCVAb, ANA titer, total IgG;

CCI [redacted]

[redacted]

[redacted]

CCI [redacted]

NOTE: If time between Screening labs and the planned Baseline visit is to exceed 12 weeks (84 days), then all Screening labs except for DMD genetic testing should be repeated and eligibility (re)confirmed prior to enrolling the subject.

- **Imaging Assessments:** NOTE: cardiac MRI should be performed after the thigh and upper limb MRI exams or conducted on different days:

  - *Thigh and upper limb MRI* (to be performed *before* functional assessments if done on the same day).

    NOTE: If time between Screening thigh and upper limb MRI and the planned Baseline visit is to exceed 12 weeks (84 days), then these should be repeated prior to enrollment.

  - *Cardiac MRI.*

    - If cardiac MRI were obtained within 3 months prior to the Screening visit, and, per the site, sponsor, and central imaging lab, the images can be submitted for central reading and are deemed adequate for confirming eligibility, then another cardiac MRI may not be required for this visit.

    - If the initial cardiac MRI cannot be analyzed (eg, due to poor imaging quality), a repeat will be required to confirm eligibility, which may take an additional 1-2 weeks. However, if quality images are unable to be obtained due to a limitation by the subject (eg, young subject unable to remain still during the scan), then echocardiogram may be permitted and read locally.

- **Functional assessments:**

  - *FOR AMBULATORY SUBJECTS*: Ambulatory status and NSAA for confirmation of eligibility;

  - *FOR NON-AMBULATORY SUBJECTS*: Ambulatory status (even if only form is completed because subject is unable to perform), pulmonary function tests (PFTs) and PUL 2.0 for confirmation of eligibility.

  NOTE: Eligibility as described in Section 4.2. If time between these functional assessments and the planned Baseline visit is to exceed 12 weeks (84 days), then these should be repeated and eligibility (re)confirmed prior to enrolling the subject.

- **Questionnaires: *C-SSRS:***

  - FOR ALL SUBJECTS AGED ⩽12 YEARS AT SCREENING: Children's Baseline/Screening (Version 6/23/10);

  - FOR ALL SUBJECTS AGED >12 YEARS AT SCREENING: Baseline/Screening (Version 1/14/09);

- **Existing symptoms and/or applicable AE monitoring.**

6.2. Visit 2 (Baseline): Day -16 (-12 to +2 days)

As described in Section 5.3.1, because 2 weeks are required to ship PF-06939926 to the site for each subject enrolled, the Baseline visit must occur at least 2 weeks prior to the Dosing visit.

In addition, even though a subject may be registered (ie, enrolled) following confirmation of eligibility during the Screening period, a repeat blood draw to confirm that the subject still has no NAb against AAV9 above the threshold per the testing laboratory will be obtained prior to the Dosing visit but will only be analyzed if required. If there were evidence of seroconversion, the subject would be discontinued from the study prior to receipt of PF-06939926.

This visit *may* be conducted over 1 or 2 days as determined by the site. Assessments during Visit 2 include:

- **Physical and neurological examination**;

- **Height and Weight** Baseline weight will be used to enroll the subject and to determine the number of investigational product vials to be shipped to the site (see above, Section 5.3.1); and below, Section 7.2.4, for guidance on obtaining height and weight measurements from non-ambulatory subjects);

- **Vital signs**: Blood pressure, pulse rate, respiratory rate, and, if clinically indicated, body temperature.

- **Clinical laboratory assessments**:

  - *Blood samples*:

  - Clinical safety, including hematology, chemistry and other labs as described in Table 1;

- **Functional Assessments:**

  - *Activity monitoring*: Activity monitor(s) may be placed on the subjects to wear just prior to the functional assessments and then continuously for the next 2 weeks;

  - *FOR AMBULATORY SUBJECTS: Ambulatory status, PFTs, 4SC, NSAA, PUL, Strength, 6MWT, in same order when possible*;

  - *FOR NON-AMBULATORY SUBJECTS: Ambulatory status, PFTs, PUL, Strength, in same order when possible.*

- **Muscle biopsies**: in those subjects in which a baseline biopsy is obtained, it will be performed under anesthesia preferably after the functional assessments. If baseline muscle biopsies have been obtained outside this study and (1) can be shipped to a sponsor's designated laboratory for use in this study, and (2) are deemed by the sponsor to be of sufficient quantity and quality, then these may replace the/ need for an additional baseline biopsy;

- **Questionnaires:** With exception of C-SSRS to confirm safety, subjects and/or caregivers can still participate in the study even if unwilling or unable to provide self-reported data:

  - *C-SSRS:*

    - FOR ALL SUBJECTS WHO WERE ≤12 YEARS OLD AT SCREENING: Children's Since Last Visit (Version 6/23/10);

    - FOR ALL SUBJECTS WHO WERE AGED >12 YEARS OLD AT SCREENING: Since Last Visit (Version 1/14/09);

- **Baseline symptoms and/or applicable AE monitoring**;
- **Concomitant medication monitoring;**
    - *Meningococcal vaccine, if applicable:* Subjects who have no contraindications and who have not previously received a MenACWY must receive at least 1 dose of a meningococcal vaccination at least 30 days prior to administration of PF-06939926, as described in Section 5.7.1.
- **Registration (ie, enrollment):** Once the subject's weight has been obtained that this visit, the site should promptly enroll the subject, which will trigger the request for investigational product shipment (Section 5.3.1).

6.3. Visit 3 (Dosing): Day 1 ±24-hour Monitoring during In-patient Stay (ie, Day 2), and Days 4, 7, and 10 (±1 Day)

Prior to dosing the subject, the investigator should ensure that the subject has not had any clinically significant infection within 30 days prior to dosing (eg, pneumonia). If a subject does develop such an infection, the Dosing visit may be re-scheduled and/or additional assessments may need to be repeated to ensure that continuation of the subject is still appropriate.

In addition, confirmation of a negative NAb test result from the Baseline visit is required in order to proceed with administration of PF-06939926 (Section 6.2).

The following assessments to be performed prior to study drug administration:

- **Vital signs:** Blood pressure, pulse rate, respiratory rate, and body temperature, within approximately 30 minutes prior to infusion;
- **Triplicate 12-lead ECG:** approximately one hour prior to infusion;
- **Clinical laboratory assessments:**
  - *Blood samples:*
    - Clinical safety, including hematology, chemistry, CCI CCI and other labs as described in Table 1, but excluding creatine kinase myocardial b fraction (CK-MB);

  - CCI [redacted]
- **≥2 mg/kg IV methylprednisolone administration** to be fully delivered between 1 and 4 hours prior to investigational drug infusion.

Dosing (*Day 1 only*):

- **Investigational product administration**. See Section 5.4.

Subjects will be monitored in-patient for at least 24 hours, and discharged on *Day 2* (or longer if deemed necessary per the investigator) as follows:

- **Vital signs**: 30 minutes, 1, 2, 4, 8, and 24 hours after the start of infusion (as described above);
- **Triplicate 12-lead ECG**: upon completion of the infusion (+30 minutes) and as clinically indicated.
- **Clinical laboratory assessments (prior to discharge and as clinically indicated):**
  - *Blood samples:* Clinical safety (Table 1), but excluding CK-MB.

  - CCI [redacted]
  - [redacted]

- **AE monitoring:** to include infusion site reaction monitoring. Subjects who experience an AE possibly related to study drug should not be discharged until the event has resolved or can be managed by subject and/or caregiver (eg, administered oral antiemetics effectively stop vomiting events);

- **Concomitant medication monitoring;**

  - *Oral prednisone* or *prednisolone*: Starting the day after the infusion of PF-06939926 (ie, Day 2), subjects will initiate oral prednisone or prednisolone at ⩾2 mg/kg/day for 2 weeks post-investigational product administration. Confirmation of compliance may be collected via a subject dosing diary, but must be specifically discussed and documented during all visits.

  - *Oral and/or IV antiemetics*: At the discretion of the investigator and/or request by the sponsor within a day following infusion of PF-06939926 (ie, Day 2), subjects may be administered antiemetics (eg, ondansetron) for approximately 7-10 days to prevent symptoms of nausea and vomiting.

Upon discharge on *Day 2* (or later if clinically indicated), subjects should be asked to stay local to the investigational site (ie, if they do not live near the investigational site, they should be asked to stay in an overnight facility) through at least the Day 14 (Week 2) visit, or longer.

Subjects will return to the site on *Days 4, 7 , and 10* (±1 day) for the following assessments:

- **Vital signs:** Blood pressure, pulse rate, respiratory rate, and body temperature;

- **Clinical laboratory assessment:**

  - *Blood sample:*

    - Clinical safety (Table 1) including CCI [redacted] but excluding CK-MB;

    - [redacted]

    - [redacted]

    - [redacted]

    - [redacted]

  NOTE: Blood and urine samples will also be collected on Days 5-9 or longer, as clinically indicated, for local laboratory testing to better understand the timing of any

changes indicative of an immune response during this period, as well as allow for increased safety monitoring and medical management to reduce the risk of adverse events.

- **AE monitoring:** to include infusion site reaction monitoring.
- **Concomitant medication monitoring,** including confirmation of compliance with increased daily oral prednisone or prednisolone prescription, as well as corresponding dosing diary, if available**.**
- **Fluid intake/output:** review with caregiver that subject has adequate fluid intake and output, as described in Section 4.5.1.

6.4. Visit 4 (Week 2): Day 14 ±1 Day

Assessments to be performed at this visit include:

- **Brief physical and neurological examination;**
- **Vital signs:** Blood pressure, pulse rate, respiratory rate, and body temperature;
- **Triplicate 12-lead ECG;**
- **Clinical laboratory assessment:**
  - *Blood sample:*
    - Clinical safety (Table 1), including CCI but excluding CK-MB;

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- **Questionnaires: *C-SSRS:***
  - FOR ALL SUBJECTS WHO WERE ≤12 YEARS OLD AT SCREENING: Children's Since Last Visit (Version 6/23/10).
  - FOR ALL SUBJECTS WHO WERE AGED >12 YEARS OLD AT SCREENING: Since Last Visit (Version 1/14/09).

- **AE monitoring**, including infusion site reaction monitoring;
- **Concomitant medication monitoring**;
  - *Oral prednisone or prednisolone:* Subjects will taper the oral prednisone or prednisolone dose to ⩾1 mg/kg/day for the remainder of the 1st month post-investigational product administration, or return to their pre-study daily doses of prednisone or prednisolone, whichever is higher. Confirmation of compliance may be collected via a subject dosing diary, but must be specifically discussed and documented during this visit.
- **Fluid intake/output:** review with caregiver that subject has adequate fluid intake and output, as described in Section 4.5.1.

6.5. Remote Visits 5, 7, 9, 11.1, 11.2, 11.3, 12.1, 12.2 (Weeks 3, 6, 10, 15, 19, 22, 32, 39): Days 21, 45, 75 ±3 Days, and Days 106, 135, 155, 225, 270 ±7 Days

The assessments at these visits may be performed remotely unless clinical concern or subject preference warrants an in-person visit. In the case of a remote visit, the site will coordinate a local phlebotomist to perform the sample collections at the subject's home.

- **Clinical laboratory assessment:**
  - *Blood sample:*
    - Clinical safety (Table 1), including CCI [redacted] CCI [redacted] excluding CK-MB (Week 3 only);
    - CCI [redacted]
    - CCI [redacted]
    - [redacted]
- **Wellness check-in**: Site staff will follow-up by contacting the subject and/or caregiver to discuss any new adverse events and/or changes to concomitant medications, including compliance with daily oral prednisone or prednisolone.
  - *Week 3 check-in will also include confirmation of adequate fluid intake and output, as described in Section 4.5.1.*
- **Activity monitoring (only at Week 39)** Activity monitors will be sent to the subject's home to wear continuously for the next 2 weeks in free-living conditions.

6.6. Visit 6 (Week 4): Day 30 ±3 Days

Assessments to be performed at this visit include:

- **Brief physical and neurological examination;**
- **Vital signs:** Blood pressure, pulse rate, respiratory rate, and body temperature;
- **Triplicate 12-lead ECG;**
- **Clinical laboratory assessment:**
  - *Blood sample*:
    - Clinical safety (Table 1), including CCI as well as re-start collection of CK-MB going forward;
    - CCI
    - CCI
    - [redacted]
- **AE monitoring, including infusion site monitoring;**
- **Concomitant medication monitoring;**
  - *Oral prednisone or prednisolone:* subjects will taper the oral prednisone or prednisolone dose to a minimum of 0.75 mg/kg/day for 1 month or return to their pre-study daily doses of prednisone or prednisolone, whichever is higher. Confirmation of compliance may be collected via a subject dosing diary, but must be specifically discussed and documented during this visit.
- **Fluid intake/output:** review with caregiver that subject has adequate fluid intake and output, as described in Section 4.5.1.

6.7. Visit 8 (Week 8): Day 60 ±3 Days

The assessments to be performed at this visit include:

- **Brief physical and neurological examination.**
- **Height and Weight;**
- **Vital signs:** Blood pressure, pulse rate, respiratory rate, and if clinically indicated, body temperature;
- **Triplicate 12-lead ECG;**

- **Clinical laboratory assessment:**
  - *Blood sample:*
    - Clinical safety (Table 1) CCI [redacted]
      [redacted]
    [redacted]
    [redacted]
  [redacted]
  [redacted]

- **Muscle biopsies**: if applicable, it will be performed under anesthesia either at this visit or the Week 12 (Day 90) visit, as determined by the sponsor and investigator;
- **AE monitoring including infusion site monitoring;**
- **Concomitant medication monitoring;**
  - *Oral prednisone or prednisolone:* subjects will taper the oral prednisone or prednisolone dose to a minimum of 0.5 mg/kg/day for 1 month or return to their pre-study daily doses of prednisone or prednisolone, whichever is higher. Confirmation of compliance may be collected via a subject dosing diary, but must be specifically discussed and documented during this visit.

6.8. Visit 10 (Week 12): Day 90 ±3 days

The assessments to be conducted at this visit include:

- **Brief physical and neurological examination;**
- **Vital signs;**
- **Clinical laboratory assessment:**
  - *Blood sample:*
    - Clinical safety (Table 1);
    [redacted]

[redacted]

- **Functional Assessments:**
  - *Activity monitoring:* Activity monitors will again be placed on the subjects to wear just prior to the functional assessments and then continuously for the next 2 weeks;
  - *FOR AMBULATORY SUBJECTS: Ambulatory status, PFTs, 4SC, NSAA, PUL, Strength, 6MWT;*
  - *FOR NON-AMBULATORY SUBJECTS: Ambulatory status, PFTs, PUL, Strength.*

  NOTE: These assessments are conducted at the site and should be assessed in the same order and preferably at around the same time of day as performed at Baseline.

[redacted]

- **Muscle biopsies**: if applicable, it will be performed under anesthesia at this visit only if not already obtained at the Week 8 (Day 60) visit, and preferably after all functional assessments (above);
- **AE monitoring;**
- **Concomitant medication monitoring;**
  - *Oral prednisone or prednisolone:* subjects will return to their pre-study glucocorticoid daily dose level, unless clinically indicated. Compliance with this daily glucocorticoid regimen must be specifically discussed and documented during this visit.

6.9. Visit 12 (Week 26): Day 180 ±7 Days

If possible and reasonable as determined by the site, this visit may be split across 2 days within the visit window, with imaging and functional assessments performed on separate days.

This visit would include the following:

- **Brief Physical Examination and Neurological Examination;**
- **Height and Weight;**
- **Vital Signs;**
- **Triplicate 12-lead ECG.**

- **Clinical laboratory assessments:**
  - *Blood samples:*
    - Clinical safety (Table 1);
    - [redacted]
    - [redacted]
  - [redacted]
  - [redacted]
  - [redacted]
- **Functional Assessments:**
  - *Activity monitoring*: Activity monitors will again be placed on the subjects to wear just prior to the functional assessments and then continuously for the next 2 weeks;
  - *FOR AMBULATORY SUBJECTS: Ambulatory status, PFTs, 4SC, NSAA, PUL, Strength, 6MWT;*
  - *FOR NON-AMBULATORY SUBJECTS: Ambulatory status, PFTs, PUL, Strength.*

  NOTE: These assessments are conducted at the site and should be assessed in the same order and preferably at around the same time of day as performed at Baseline.

[redacted]

- **Muscle biopsies**: if applicable, it will be performed under anesthesia either at this visit or the Week 52 (Day 360) visit, as determined by the sponsor and investigator, and preferably after all imaging and functional assessments (above);
- **AE monitoring;**
- **Concomitant medication monitoring,** including compliance with the required daily glucocorticoid regimen.

6.10. Visit 13 (Week 52): Day 360 ±7 Days

If possible and reasonable as determined by the site, this visit may be split across 2 or 3 days within the visit window, with imaging and functional assessments performed on separate days.

This visit would include the following:

- **Brief Physical Examination and Neurological Examination;**
- **Height and Weight;**
- **Vital Signs;**
- **Triplicate 12-lead ECG;**
- **Clinical laboratory assessments:**
  - *Blood samples:*
    - Clinical safety (Table 1);
    - [redacted]
    - [redacted]
  - [redacted]
  - [redacted]
- **Imaging Assessments:**
  - [redacted]
  - *Cardiac MRI* should be performed after thigh and upper limb MRI or on different days. If the cardiac MRI is unable to be evaluated due to quality, it will either be repeated or, if necessary, substituted by a locally-read echocardiogram.
- **Functional Assessments:**
  - *Activity monitoring:* Activity monitors will again be placed on the subjects to wear just prior to the functional assessments and then continuously for the next 2 weeks;
  - *FOR AMBULATORY SUBJECTS: Ambulatory status, PFTs, 4SC, NSAA, PUL, Strength, 6MWT;*
  - *FOR NON-AMBULATORY SUBJECTS: Ambulatory status, PFTs, PUL, Strength.*

NOTE: These assessments are conducted at the site and should be assessed in the same order and preferably at around the same time of day as performed at Baseline.

[redacted]

- **Muscle biopsies**: if applicable, it will be performed under anesthesia at this visit only if not already obtained at the Week 26 (Day 180) visit, and preferably after the imaging and functional assessments (above);
- **AE monitoring.**
- **Concomitant medication monitoring,** including compliance with the required daily glucocorticoid regimen**.**

6.11. Visits 14, 15, 16, 17 (Long-term Follow-up Phase Years 2-5): Annually - Days 730, 1095, 1460, 1825 ±30 Days *or* Early Termination

If possible and reasonable as determined by the site, this visit may be split across 2 or 3 days within the visit window, with imaging and functional assessments performed on separate days.

This visit would include the following:

- **Brief Physical Examination and Neurological Examination;**
- **Height and Weight;**
- **Vital Signs;**
- **Triplicate 12-lead ECG;**
- **Clinical laboratory assessments:**
  - *Blood samples:*
    - Clinical safety (Table 1);

[redacted]

[redacted]

- **Muscle biopsies**: will be performed under anesthesia preferably after the imaging and functional assessments (below), but only in the event of an Early Termination visit *and* with subject/caregiver consent provided that the subject has not yet completed the maximum of 3 procedures. No subject will undergo biopsies at more than 3 total visits.

- **Imaging Assessments:**

  [redacted]

  - *Cardiac MRI* should be performed after thigh and upper limb MRI or on different days. If the cardiac MRI is unable to be evaluated due to quality, it will either be repeated or, if necessary, substituted by a locally-read echocardiogram.

- **Functional Assessments:**

  - *Activity monitoring:* Activity monitors will again be placed on the subjects to wear just prior to the functional assessments and then continuously for the next 2 weeks;

  - *FOR AMBULATORY SUBJECTS: Ambulatory status, PFTs, 4SC, NSAA, Strength, PUL, 6MWT;*

  - *FOR NON-AMBULATORY SUBJECTS: Ambulatory status, PFTs, PUL, Strength.*

  NOTE: These assessments are conducted at the site and should be assessed in the same order and preferably at around the same time of day as performed at Baseline.

[redacted]

- **AE monitoring;**

- **Concomitant medication monitoring.**

6.12. Remote Visits 13.1, 14.1, 15.1, 16.1 (Long-term Follow-Up Phase Years 1.5, 2.5, 3.5, 4.5): Days 545, 910, 1275, 1640 ±30 Days

The assessments at these visits may be performed remotely unless clinical concern or subject preference warrants an in-person visit. In the case of a remote visit, the site will coordinate a local phlebotomist to perform the sample collections at the subject's home.

- **Clinical laboratory assessment:**
  - *Blood sample:*
    - Clinical safety (Table 1);
    - [redacted]
- **Wellness check-in**: Site staff will follow-up by contacting the subject and/or caregiver to discuss any new adverse events and/or changes to concomitant medications.

6.13. Subject Withdrawal

6.13.1. Withdrawal of Consent

Subjects will receive only one dose of investigational product. However, given the nature of gene transfer, the gene remains in the body for a long period of time. Therefore, subjects are followed for 5 years post administration of PF-06939926. The only exception to this is when a subject or parent or legal guardian on behalf of the subject specifically withdraws consent for any further contact with him or her or persons previously authorized by the subject to provide this information. Subjects or parent(s) or legal guardian(s) should notify the investigator in writing of the decision to withdraw consent from future follow-up, whenever possible. The withdrawal of consent should be explained in detail in the medical records by the investigator, as to whether the withdrawal is from study procedures and/or post-treatment study follow-up, and entered on the appropriate CRF page. In the event that vital status (whether the subject is alive or dead) is being measured, publicly available information should be used to determine vital status only as appropriately directed in accordance with local law.

Subjects may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator or sponsor for safety (see also the Withdrawal From the Study Due to Adverse Events Section 8.1.3) or behavioral reasons, or the inability of the subject to comply with the protocol-required schedule of study visits or procedures at a given study site.

If a subject does not return for a scheduled visit, every effort should be made to contact the subject. All attempts to contact the subject and information received during contact attempts must be documented in the subject's medical record. In any circumstance, every effort should be made to document subject outcome, if possible. The investigator should inquire about the reason for withdrawal, request that the subject return for a final visit, if applicable, and follow up with the subject regarding any unresolved adverse events (AEs).

It may be appropriate for the subject to return to the clinic for final safety assessments and to be questioned regarding their reason for withdrawal. The Early Termination assessments may be performed according to the Schedule of Activities and Section 6.11.

If the subject withdraws from the study, and also withdraws consent for disclosure of future information, no further evaluations should be performed, and no additional data should be collected. The sponsor may retain and continue to use any data collected before such withdrawal of consent.

6.13.2. Lost to Follow-up

All reasonable efforts must be made to locate subjects to determine and report their ongoing status. This includes follow-up with persons authorized by the subject as noted above. Lost to follow-up is defined by the inability to reach the subject after a minimum of 2 documented phone calls, faxes, or e-mails as well as lack of response by the subject to 1 registered mail letter. All attempts should be documented in the subject's medical records. If it is determined that the subject has died, the site will use locally permissible methods to obtain the date and cause of death. If the investigator's use of a third-party representative to assist in the follow-up portion of the study has been included in the subject's informed consent, then the investigator may use a sponsor-retained third-party representative to assist site staff with obtaining the subject's contact information or other public vital status data necessary to complete the follow-up portion of the study. The site staff and representative will consult publicly available sources, such as public health registries and databases, in order to obtain updated contact information. If, after all attempts, the subject remains lost to follow-up, then the last-known-alive date as determined by the investigator should be reported and documented in the subject's medical records.

7. STUDY ASSESSMENTS

Every effort should be made to ensure that the protocol-required tests and procedures are completed as described. However, it is anticipated that from time to time there may be circumstances, outside of the control of the investigator that may make it unfeasible to perform the test. In these cases, the investigator will take all steps necessary to ensure the safety and well-being of the subject. When a protocol-required test cannot be performed, the investigator will document the reason for this and any corrective and preventive actions that he or she has taken to ensure that normal processes are adhered to as soon as possible.

The sponsor study team will be informed of these incidents in a timely manner.

In the event that a subject becomes intolerant to MRI scanning (eg, loses ambulation), the subject may be requested to complete cardiac MRI (or echocardiogram) only, and upper limb

MRI when possible. Other assessments may be modified or deemed not applicable to non-ambulatory subjects, as described below.

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7.1. Blood Volume

While frequent blood collection, particularly within the first few months after receiving PF-06939926, is critical to ensure subject safety in this study, blood volume limits have been set for this pediatric patient population according to those described in the Bulletin of the World Health Organization.[66] In accordance with these recommendations, compiled from 5 studies and 9 sets of guidelines, blood sampling should not exceed 5% based on an estimated total blood volume of 75-80 mL/kg within a 24-hour period, or 10% of this volume within an 8-week period, even at the minimum weight required for participation in this study (ie, 15 kg). CCI [redacted]

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7.2. Safety

7.2.1. Clinical Laboratory

The following safety laboratory tests will be performed at times defined in the Schedule of Activities section of this protocol. Additional laboratory values may be reported as a result of the method of analysis or type of analyzer used by the clinical laboratory, or as derived from calculated values. Such tests would not require additional collection of biospecimens. Unscheduled clinical labs may be obtained at any time during the study to assess any perceived safety concerns. If a subject has a peripherally inserted central catheter (PICC) line, it must only be used for blood collections and not for study drug administration.

Detailed collection, processing, storage, and shipment instructions will be provided in the central laboratory manual.

Table 1. Central Laboratory Tests

Hematology	Chemistry	Urinalysis	Other
Hemoglobin Hematocrit Red blood cell (RBC) count Platelet count White blood cell count (and morphology as applicable) Total neutrophils (Abs) Absolute neutrophils (ANC) Eosinophils (Abs) Monocytes (Abs) Basophils (Abs) Lymphocytes (Abs) Red blood cell indices (mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration) Haptoglobin[c]	BUN and Creatinine Glucose Calcium Sodium Potassium Magnesium[a] Chloride Total CO_2 (Bicarbonate) AST, ALT, Total Bilirubin (direct and indirect bilirubin) Alkaline phosphatase Uric acid Albumin Total protein Serum Phosphorus Cystatin C Creatine kinase Creatine kinase myocardial b fraction (CK-MB)[d] Amylase Lipase GGT C-reactive protein (CRP) Cardiac troponin I	Dipstick Analysis: pH Glucose (qual) Protein (qual) Blood (qual) Ketones Nitrites Leukocyte esterase Microscopy[b]	PT aPTT Glutamate dehydrogenase (GLDH) CCI [redacted] **Immunogenicity:** CCI [redacted] mini-dystrophin ADA anti-virus immune response, binding Ab **<u>For screening only</u>**: DMD genetic test (if required to confirm DMD diagnosis) International normalized ratio (INR); Hepatitis A virus (anti-HAV) immunoglobulin M Hepatitis B antigen Hepatitis C antibody, anti-nuclear antibody, total CCI [redacted]
Additional Tests (repeats for any potential cases of drug-induced liver injury)			
AST, ALT (repeat) Total bilirubin (repeat) Albumin (repeat) Alkaline phosphatase (repeat) Direct bilirubin Indirect bilirubin Creatine kinase CK-MB GGT GLDH PT			
Genetic Testing for a-HUS-Central Laboratory for subjects who experience an event compatible with aHUS			

Table 1. Central Laboratory Tests

a. Magnesium will be collected on Day 1, Day 7, and Day 14.
b. Only if urine dipstick is positive for blood, protein, nitrites or leukocyte esterase.
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d. CK-MB will be collected at all planned visits with the exception of Dosing Day 1 until Week 4, at which point it will be collected at all planned visits for the remainder of study participation.

Biospecimen samples will also be collected for local laboratory testing as described in the Schedule of Activities. At a minimum these will include hematology, chemistry and urinalysis testing.

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7.2.2. Physical Examinations

Physical examinations may be conducted by a physician, trained physician's assistant, or nurse practitioner as acceptable according to local regulation. The full physical examination will include head, ears, eyes, nose, mouth, skin, heart and lung examinations, lymph nodes, gastrointestinal, and musculoskeletal systems. Abnormal findings will be further characterized as "clinically significant" or "not clinically significant" for the purposes of reporting as adverse events.

7.2.2.1. Brief Physical Examinations

Only brief physical examinations will be performed post-baseline unless safety concerns warrant full examination. These may be conducted by a physician, trained physician's assistant, or nurse practitioner as acceptable according to local regulation. The brief physical examination will include general appearance, chest (heart and lungs), abdomen, musculoskeletal (joints, tenderness, presence of new fractures, joint contractures).

7.2.3. Neurological Examination

Only brief neurological examinations will be performed post-baseline unless safety concerns warrant full examination. These will be conducted by a neurologist. The neurological examination will include Mental Status, cranial nerves II-XII, motor (upper and lower limb bulk strength), deep tendon reflexes, sensory testing, station and gait, and coordination.

FOR NON-AMBULATORY SUBJECTS: Only those domains of the neurological examination deemed safe and appropriate for non-ambulatory subjects will be performed, as determined by the examiner (eg, exclusion of station and gait).

7.2.3.1. Brief Neurological Examination

A brief neurological examination will be conducted by a neurologist. The brief neurological examination will include motor (bulk strength, right and left separately, both upper and lower limbs separately) and station and gait.

FOR NON-AMBULATORY SUBJECTS: As above, domains of this examination will only be performed if they are deemed safe and appropriate.

7.2.4. Height and Weight Measurements

Preferably both height and weight measurements will be collected in the morning.

7.2.4.1. Height

FOR NON-AMBULATORY SUBJECTS: If a subject is unable to stand safely, an estimated height can be calculated using the subject's ulnar length and the instructions provided in the Functional Assessment Manual.

7.2.4.2. Weight

For measuring weight, a calibrated scale with appropriate range and resolution is to be used and must be placed on a stable, flat surface. Subjects must remove shoes, bulky layers of clothing, and jackets so that only light clothing remains. They must also remove the contents of their pockets and remain still during measurement of weight.

FOR NON-AMBULATORY SUBJECTS: Body weights may be measured using a calibrated chair scale or wheelchair scale (eg, subtract the wheelchair weight from the total weight), or other method with agreement from the sponsor, since body weight is required to determine the dose of PF-06939926 for each subject. To obtain as accurate a measurement as possible, all other contents besides the subject (eg, blankets, items in chair pockets, etc.) should be removed from the wheelchair before rolling onto the scale.

Preferably both height and weight measurements will be collected in the morning.

7.2.5. Vital Signs

Respiratory rate should be measured after approximately 5 minutes rest by observing and counting the respirations of the subject for 30 seconds and multiplied by 2. Preferably, respiratory rate will be obtained just before blood pressure measurement, and ideally the position of the subject during the measurement (e.g., sitting or supine) will be consistent for each collection from a given subject.

Blood pressure, pulse rate, and respiratory rate will be measured at times specified in Schedule of Activities section of this protocol. Unscheduled collection times will be permitted, as necessary, to ensure appropriate collection of safety data.

Blood pressure will be measured with the subject's arm supported at the level of the heart, and recorded to the nearest mm Hg after at least 5 minutes of rest. Whenever possible, the same arm (preferably the dominant arm) should be used throughout the study, and, ideally,

the measurement should be taken while the subject is in the same position (eg, sitting or supine).

Wherever possible, the same size blood pressure cuff, which has been properly sized and calibrated, will be used to measure blood pressure each time. The use of automated devices for measuring BP and pulse rate are acceptable, although, when done manually, pulse rate will be measured in the brachial/radial artery for at least 30 seconds.

Body temperature will also be collected at the times specified in the Schedule of Activities section of this protocol, and as clinically indicated.

7.2.6. Triplicate Electrocardiogram (ECG)

ECGs should be collected in triplicate at times specified in the Schedule of Activities section of this protocol.

All scheduled ECGs should be performed after the subject has rested quietly for at least 10 minutes in a supine position.

To ensure safety of the subjects, a qualified individual at the investigator site will make comparisons to baseline measurements. The QTc interval should be recorded using the QTcF (QTc Fridericia) value in milliseconds.

If the QTc interval is increased by ⩾45 msec from the baseline, or an absolute QTc value is ⩾500 msec for any scheduled ECG, then 2 additional ECGs will be collected, approximately 2-4 minutes apart, to confirm the original measurement. If either of the QTc values from these repeated ECGs remains above the threshold value (⩾45 msec from the baseline, which for this study would be on Day 1, prior to receiving PF-06939926; or is ⩾500 msec), then a single ECG must be repeated at least hourly until QTc values from 2 successive ECGs fall below the threshold value that triggered the repeat measurement.

If QTcF values remain ⩾500 msec (or ⩾45 msec from the baseline) for greater than 4 hours (or sooner at the discretion of the investigator); or QTcF intervals get progressively longer, the subject should undergo continuous ECG monitoring. A cardiologist should be consulted if QTcF intervals do not return to less than 500 msec (or to <45 msec above the baseline) after 8 hours of monitoring (or sooner at the discretion of the investigator).

In some cases, it may be appropriate to repeat abnormal ECGs to rule out improper lead placement as contributing to the ECG abnormality. It is important that leads are placed in the same positions each time in order to achieve precise ECG recordings. If a machine-read QTcF value is prolonged, as defined above, repeat measurements may not be necessary if a qualified physician's interpretation determines that the QTcF values are in the acceptable range.

7.2.7. Safety Imaging

7.2.7.1. Management of Incidental Findings

An incidental finding is one unknown to the subject that has potential health or reproductive importance, which is discovered unexpectedly in the course of a research study, but is unrelated to the purpose and beyond the aims of the study.

MRI will be reviewed by a central review facility. The purpose of this review is to evaluate images for muscle changes or cardiac safety. Central image review is not a complete medical review of the subject. If during the central review process, an unexpected observation is identified and this finding could, in the opinion of the central reviewer, have a significant health or reproductive consequence, this finding may be shared with the study sponsor for disclosure to the principal investigator. All follow-up testing and final diagnosis will be left to the discretion of the medical professionals at the site or those with an existing physician-patient relationship. The principal investigator will be responsible for reporting any adverse events identified from incidental findings as described in the Section 8. Identification of such incidental findings during the central review process should not be expected, and the site maintains responsibility of performing a general safety review of all images as per site protocols.

7.2.7.2. Cardiac MRI (or Echocardiogram, if necessary)

Cardiac MRI should be collected at times specified in the Schedule of Activities section of this protocol.

Cardiac MRI with gadolinium is the preferred method for cardiac imaging, when possible. When cardiac MRI with gadolinium is used, it should only be collected after thigh and upper limb MRI is complete. Otherwise cardiac MRI should be done on a different visit day from the thigh and upper limb MRI. If the subject has a contraindication to gadolinium, cardiac MRI without gadolinium will be acceptable. Cardiac MRI will be read by a central imaging vendor and only the results of the LVEF will be provided to sites.

FOR AMBULATORY SUBJECTS: If the LVEF falls below 50% on the cardiac MRI at any follow-up visit, the subject should be referred to a cardiologist for further assessment.

FOR NON-AMBULATORY SUBJECTS: For those non-ambulatory subjects for whom the LVEF is already <50% at Screening, documentation of consultation with a cardiologist at least annually should be kept on file as part of standard of care.

Sites will be provided with a scanning and image transmittal guide for collection of the cardiac MRI.

If the cardiac MRI is deemed unable to be evaluated and a repeat MRI is unlikely to resolve the issue (eg, if subject is unable to remain still in the scanner), a locally-read echocardiogram may be an acceptable substitute. To ensure safety of the subjects, a qualified individual at the site will evaluate the echocardiogram at least for LVEF, but other parameters may also be assessed, such as ventricular systolic pressure, left arterial diameter,

left ventricular mass index, left ventricular end diastolic diameter, left ventricular end systolic diameter, shortening fraction, left ventricular posterior wall thickness, tricuspid valvular regurgitation presence and pericardial effusion.

7.2.8. Suicidal Ideation and Behavior Assessment

FOR ALL SUBJECTS AGED ≤12 YEARS AT SCREENING: The Columbia Suicide Severity Rating Scale (C-SSRS) Children's Baseline/Screening (Version 6/23/10) will be completed at Screening and the C-SSRS: Children's Since Last Visit (Version 6/23/10) will be evaluated at the subsequent times specified in the Schedule of Activities. Given the sensitive nature of the subject matter of this assessment, the C-SSRS will be conducted with the subject's care giver/legal guardian on the subject's behalf throughout the study for subjects who are 12 years of age and younger at Screening, rather than administering this evaluation directly with these study subjects.

FOR ALL SUBJECTS AGED >12 YEARS AT SCREENING: The C-SSRS Baseline/Screening version (Version 1/14/09) will be completed at Screening, covering both lifetime and the past 6 months/0.5 year, and the C-SSRS Since Last Visit (Version 1/14/09) will be evaluated at the subsequent times specified in the Schedule of Activities. Subjects >12 years old at Screening will complete these assessments themselves. However, upon request, site staff may support the physical completion of this assessment on behalf of the subject if, for example, a subject is unable to record responses due to loss of upper limb function.

At Screening, if the subject endorses a 4 or 5 on the C-SSRS ideation section or reports any suicidal behavior, then the subject is not eligible for study participation and an evaluation of suicide risk (risk assessment) must be completed.

Risk Assessment: In the event that a subject endorses a 4 or 5 on the C-SSRS ideation section or reports any suicidal behavior, an evaluation of suicide risk (risk assessment) will be completed as part of the psychiatric evaluation and assessment of subject safety to participate will be performed by the following child and adolescent mental health provider (MHP): In the US: 1) Child and Adolescent Psychiatrists (board certified or board eligible), 2) psychiatrist who have training and experience in the diagnosis and treatment of psychiatric disorders in the pediatric population, or 2) Psy. D. or Ph.D. level Clinical Psychologists, licensed Master's level Clinical Social Workers (MSW) or licensed Psychiatric Nurse Practitioners (PNP) who have training and experience in the diagnosis and treatment of psychiatric disorders in the pediatric population.

Written documentation of the risk assessment should be included in the subject's source documentation and the risk assessment CRF will be completed. The risk assessment CRF serves as further verification that the psychiatric evaluation and assessment of subject safety have been completed for all subjects endorsing items 4 or 5 on the C-SSRS ideation section or reporting suicidal behavior.

Additional details regarding these assessments, including training and any additional requirements, will be provided in the Clinical Outcomes Assessment Manual.

7.2.9. Concomitant Medication Monitoring

See Section 5.7.

7.2.9.1. Subject Dosing Diary

Subjects or caregivers on behalf of subjects may be required to complete dosing diaries to ensure compliance with the increased and then tapering dose levels of daily oral prednisone or prednisolone required for at least the first 3 months post-dose of PF-06939926. Since these diaries were designed to be completed electronically, if not used, medication compliance, particularly with the required daily dose of glucocorticoids through the first year, is expected to at least be reviewed with the subject and/or caregiver and documented at all in-clinic and remote visits (ie, wellness check-ins).

7.2.10. Infusion Site Monitoring

From the initiation of the IV infusion and then at the times described in the Schedule of Activities, subjects will be monitored for signs of any infusion site reactions including but not limited to erythema, swelling, bruising, pain or pruritus.

7.2.11. Wellness Check-in

Subjects will be contacted by the site at defined time-points and intervals as described in the Schedule of Activities, and as clinically indicated, to collect information on any adverse events experienced and/or updates to concomitant medications since the last contact with the subject. These may also include confirmation of adequate fluid intake and output, as described in Section 4.5.1. Written documentation of the Wellness Check-in will be included in the subjects' source documentation and any adverse events or concomitant medication changes will be captured in the associated CRFs.

7.2.12. Triggers for Additional Safety Monitoring

Should any of the following conditions be observed, the subject should be brought to the investigational site as soon as possible and within 24 hours for evaluation with the investigator or designated medically qualified individual:

- No urine output for 24 hours and/or evidence of significant reduction in estimated glomerular filtration rate, such as determined by increases in serum cystatin C;
- [redacted]
- Abnormality on blood smear (indicating unequivocal hemolysis);
- Platelet count <100 GI/L on repeat (ie, ×2).

Hospital admission and/or treatment with rescue medication, as per Section 5.8, may be warranted, as per the investigator and medical team's and/or sponsor study team's discretion.

Cardiac Troponin I

For the first value >17 × ULN, and for any value >3× the most recent value:

- Perform clinical evaluation for cardiac signs or symptoms;
- Repeat cTnI test within 24-48 hours after the result was received;
- If cTnI remains persistently elevated or if signs or symptoms are observed or reported: perform ECG & consider cardiac consult.

For any value >33 × ULN, unless such elevation has occurred and been evaluated in the last 3 months:

- Perform clinical evaluation for cardiac signs or symptoms;
- Repeat cTnI test within 24-48 hours after the result was received;
- Perform ECG and consider cardiac consult.

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[redacted]

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[redacted]

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7.6. Muscle Biopsies

In order to evaluate the expression and distribution of mini-dystrophin produced post-gene transfer relative to baseline, muscle biopsies will be collected according to the Schedule of Activities. CCI

Open muscle biopsies will be taken from the bicep brachii, and needle biopsies may be taken from other muscle groups, such as gastrocnemius and vastus lateralis, for each assessment. No subject will undergo biopsies at more than 3 total visits. The biopsies will be conducted by a qualified surgeon with adequate experience with this specific procedure, as confirmed and approved by the sponsor beforehand. This procedure will be performed using anesthesia (eg, regional block or under general anesthesia) according to institutional standards and specific instructions regarding post-operative care will also be provided to the subjects after the procedure.

Unless instructed by Pfizer to site staff in writing, all subjects will undergo open muscle biopsies taken from the bicep brachii at Baseline and at approximately 2 and 12 months following receipt of PF-06939926. Examples of explicit alternative instructions provided by Pfizer to site staff regarding biopsies for a given subject include the following:

- *FOR AMBULATORY SUBJECTS:* Sites will be informed if needle biopsies will be collected in addition to open muscle biopsies for a particular subject at a specific timepoint, as well as from which specific muscle group(s).

- *FOR NON-AMBULATORY SUBJECTS:* For non-ambulatory subjects, only open muscle biopsies from the biceps brachii will be collected, and the option to reduce the total number of biopsies will be considered. Sites will be informed if fewer biopsies will be collected for a given subject, as well as the timepoints for collection.

Detailed collection, processing, storage, and shipment instructions will be provided in the biopsy and central laboratory manuals. Additional training on the aforementioned procedures will be provided by the sponsor or sponsor representative.

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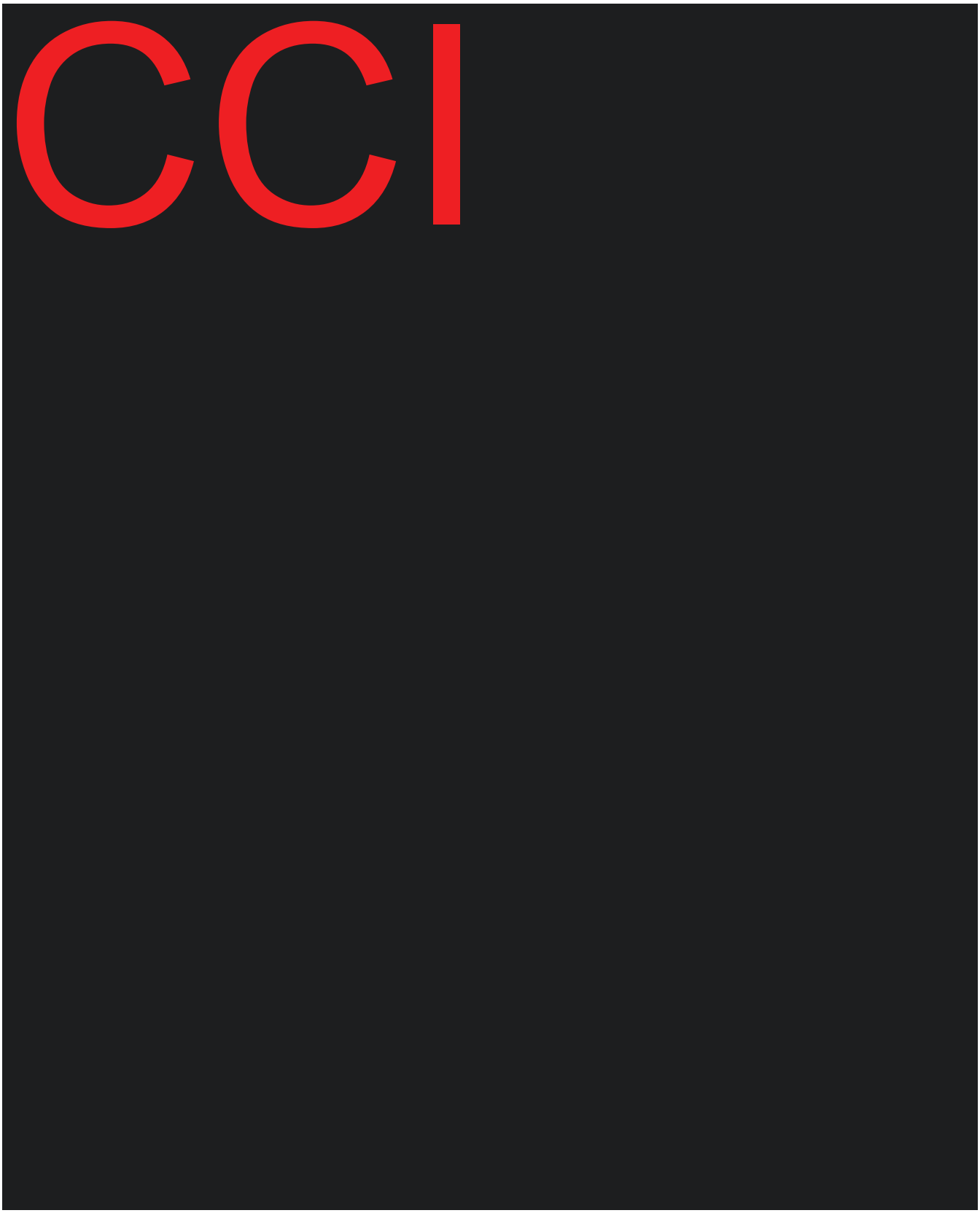
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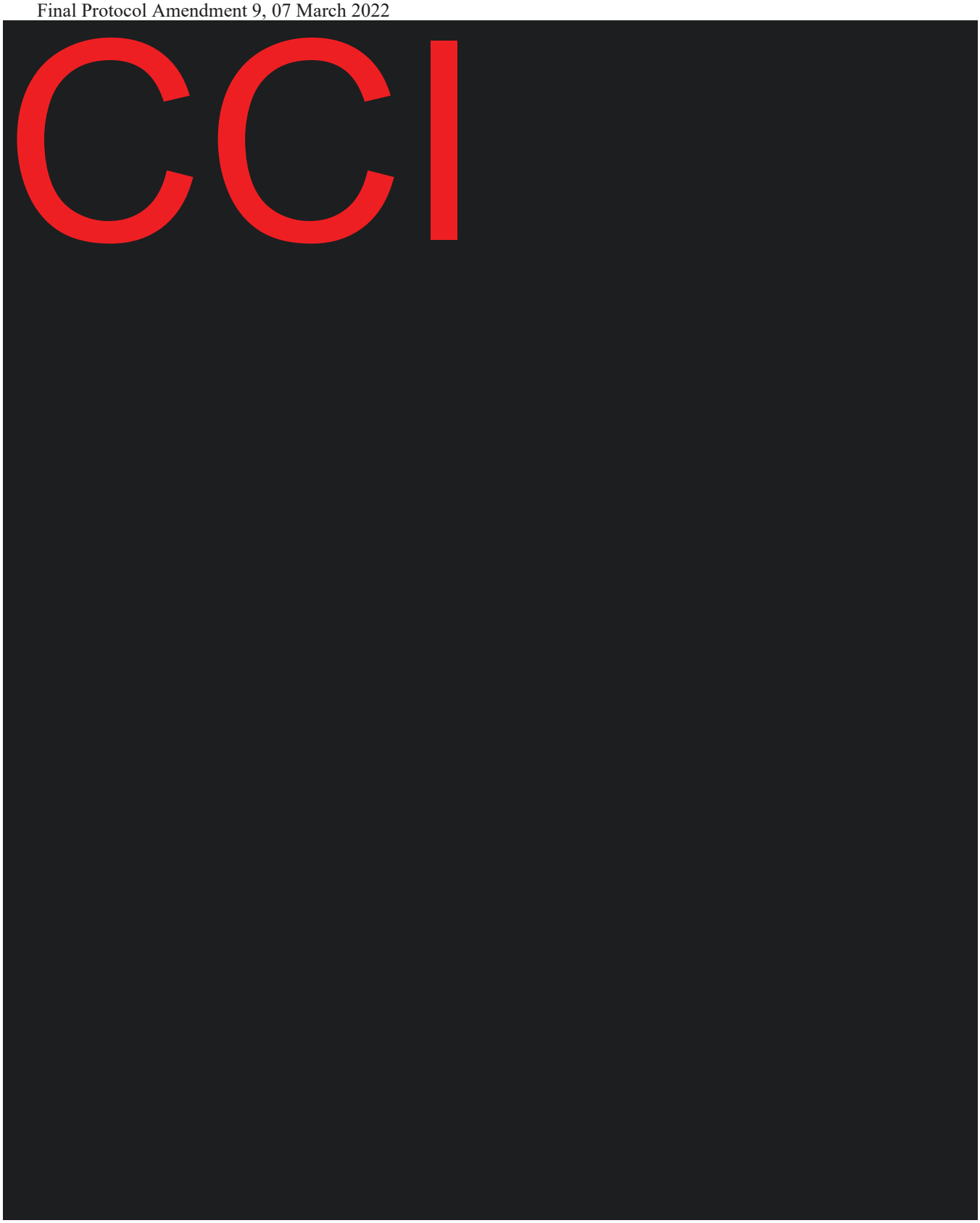
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8. ADVERSE EVENT REPORTING

8.1. Requirements

The table below summarizes the requirements for recording safety events on the CRF and for reporting safety events on the Clinical Trial (CT) Serious Adverse Event (SAE) Report Form to Pfizer Safety. These requirements are delineated for 3 types of events: (1) SAEs; (2) non-serious adverse events (AEs); and (3) exposure to the investigational product under study during pregnancy or breastfeeding, and occupational exposure.

Safety Event	**Recorded on the CRF**	**Reported on the CT SAE Report Form to Pfizer Safety Within 24 Hours of Awareness**
SAE	All	All
Non-serious AE	All	None
Exposure to the investigational product under study during pregnancy or breastfeeding, and occupational exposure	All (regardless of whether associated with an AE), **except occupational exposure**	Exposure during pregnancy, exposure via breastfeeding, occupational exposure (regardless of whether associated with an AE)

All observed or volunteered events regardless of treatment group or suspected causal relationship to the investigational product(s) will be reported as described in the following paragraphs.

Events listed in the table above that require reporting to Pfizer Safety on the CT SAE Report Form within 24 hours of awareness of the event by the investigator **are to be reported regardless of whether the event is determined by the investigator to be related to an investigational product under study**. In particular, if the SAE is fatal or life-threatening, notification to Pfizer Safety must be made immediately, irrespective of the extent of available event information. This time frame also applies to additional new (follow-up) information on previously forwarded reports. In the rare situation that the investigator does not become immediately aware of the occurrence of an event, the investigator must report the event within 24 hours after learning of it and document the time of his/her first awareness of the event.

For each event, the investigator must pursue and obtain adequate information both to determine the outcome and to assess whether it meets the criteria for classification as an SAE (see the Serious Adverse Events section below). In addition, the investigator may be requested by Pfizer Safety to obtain specific follow-up information in an expedited fashion. This information is more detailed than that recorded on the CRF. In general, this will include a description of the event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Any information relevant to the event, such as concomitant medications and illnesses, must be provided. In the case of a subject death, a summary of available autopsy findings must be submitted as soon as possible to Pfizer Safety. Any pertinent additional information must be reported on the CT SAE Report Form; additional source documents (eg, medical records, CRF, laboratory data) are to be sent to Pfizer Safety **ONLY** upon request.

As part of ongoing safety reviews conducted by the sponsor, any non-serious AE that is determined by the sponsor to be serious will be reported by the sponsor as an SAE. To assist in the determination of case seriousness, further information may be requested from the investigator to provide clarity and understanding of the event in the context of the clinical study.

As noted in the Protocol-Specified Serious Adverse Events section, should an investigator judge one of the identified protocol-specified SAEs to have a causal relationship with the investigational product, the investigator must report the SAE to Pfizer Safety within 24 hours of investigator awareness.

8.1.1. Additional Details on Recording Adverse Events on the CRF

All events detailed in the table above will be recorded on the AE page(s) of the CRF. It should be noted that the CT SAE Report Form for reporting of SAE information is not the same as the AE page of the CRF. When the same data are collected, the forms must be completed in a consistent manner. AEs should be recorded using concise medical terminology and the same AE term should be used on both the CRF and the CT SAE Report Form for reporting of SAE information.

8.1.2. Eliciting Adverse Event Information

The investigator is to record on the CRF all directly observed AEs and all AEs spontaneously reported by the study subject/parent(s)/legal guardian/legally acceptable representative. In addition, each study subject/parent(s)/legal guardian/legally acceptable representative will be questioned about the occurrence of AEs in a non-leading manner.

8.1.3. Withdrawal from the Study Due to Adverse Events (see also the Subject Withdrawal section)

Withdrawal due to AEs should be distinguished from withdrawal due to other causes, according to the definition of AE noted below, and recorded on the CRF.

When a subject withdraws from the study because of an SAE, the SAE must be recorded on the CRF and reported, as appropriate, on the CT SAE Report Form, in accordance with the Requirements section above.

8.1.4. Time Period for Collecting AE/SAE Information

The time period for actively eliciting and collecting AEs and SAEs ("active collection period") for each subject begins from the time the subject provides informed consent, which is obtained before the subject's participation in the study (ie, before undergoing any study-related procedure and/or receiving investigational product), through and including a minimum of 1 year following administration of the investigational product, also referred to as the Treatment Phase. In the Long-term Follow-up Phase (ie, Years 2 through 5), only SAEs and any AEs believed to be related to the investigational product will be reported.

For subjects who are screen failures, the active collection period ends when screen failure status is determined.

8.1.4.1. Reporting SAEs to Pfizer Safety

All SAEs occurring in a subject during the active collection period are reported to Pfizer Safety on the CT SAE Report Form.

SAEs occurring in a subject after the active collection period has ended are reported to Pfizer Safety if the investigator becomes aware of them; at a minimum, all SAEs that the investigator believes have at least a reasonable possibility of being related to investigational product must be reported to Pfizer Safety.

Follow up by the investigator continues throughout and after the active collection period and until the event or its sequelae resolve or stabilize at a level acceptable to the investigator, and Pfizer concurs with that assessment.

8.1.4.2. Recording Non-serious AEs and SAEs on the CRF

During the active collection period, both non-serious AEs and SAEs are recorded on the CRF.

Follow-up by the investigator may be required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator, and Pfizer concurs with that assessment.

8.1.5. Causality Assessment

The investigator's assessment of causality must be provided for all AEs (serious and non-serious); the investigator must record the causal relationship on the CRF, and report such an assessment in accordance with the SAE reporting requirements, if applicable. An investigator's causality assessment is the determination of whether there exists a reasonable possibility that the investigational product caused or contributed to an AE; generally the facts (evidence) or arguments to suggest a causal relationship should be provided. If the investigator does not know whether or not the investigational product caused the event, then the event will be handled as "related to investigational product" for reporting purposes, as defined by the sponsor. If the investigator's causality assessment is "unknown but not related" to investigational product, this should be clearly documented on study records.

In addition, if the investigator determines that an SAE is associated with study procedures and/or concomitant medications or therapies, the investigator must record this causal relationship in the source documents and CRF, and report such an assessment in the dedicated section of the CT SAE Report Form and in accordance with the SAE reporting requirements.

8.1.6. Sponsor's Reporting Requirements to Regulatory Authorities

AE reporting, including suspected unexpected serious adverse reactions, will be carried out in accordance with applicable local regulations.

8.2. Definitions

8.2.1. Adverse Events

An AE is any untoward medical occurrence in a study subject administered a product or medical device; the event need not necessarily have a causal relationship with the treatment or usage. Examples of AEs include, but are not limited to:

- Abnormal test findings;
- Clinically significant signs and symptoms;
- Changes in physical examination findings;
- Hypersensitivity;
- Progression/worsening of underlying disease;
- Drug abuse;
- Drug dependency.

Additionally, AEs may include signs and symptoms resulting from:

- Drug overdose;
- Drug withdrawal;
- Drug misuse;
- Drug interactions;
- Extravasation;
- Exposure during pregnancy (EDP);
- Exposure via breastfeeding;
- Medication error;
- Occupational exposure.

8.2.2. Abnormal Test Findings

Abnormal objective test findings should be recorded as AEs when any of the following conditions are met:

- Test result is associated with accompanying symptoms; and/or
- Test result requires additional diagnostic testing or medical/surgical intervention; and/or
- Test result leads to a change in study dosing (outside of any protocol-specified dose adjustments) or discontinuation from the study, significant additional concomitant drug treatment, or other therapy; and/or
- Test result is considered to be an AE by the investigator or sponsor.

Merely repeating an abnormal test, in the absence of any of the above conditions, does not constitute an AE. Any abnormal test result that is determined to be an error does not require recording as an AE.

8.2.3. Serious Adverse Events

A serious adverse event is any untoward medical occurrence at any dose that:

- Results in death;
- Is life-threatening (immediate risk of death);

- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity (substantial disruption of the ability to conduct normal life functions);
- Results in congenital anomaly/birth defect.

Or that is considered to be:

- An important medical event.

Medical and scientific judgment is exercised in determining whether an event is an important medical event. An important medical event may not be immediately life-threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the subject or may require intervention to prevent one of the other AE outcomes, the important medical event should be reported as serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

8.2.4. Hospitalization

Hospitalization is defined as any initial admission (even less than 24 hours) in a hospital or equivalent healthcare facility, or any prolongation of an existing admission. Admission also includes transfer within the hospital to an acute/intensive care unit (eg, from the psychiatric wing to a medical floor, medical floor to a coronary care unit, or neurological floor to a tuberculosis unit). An emergency room visit does not necessarily constitute a hospitalization; however, the event leading to the emergency room visit is assessed for medical importance.

Hospitalization does not include the following:

- Rehabilitation facilities;
- Hospice facilities;
- Respite care (eg, caregiver relief);
- Skilled nursing facilities;
- Nursing homes;
- Same-day surgeries (as outpatient/same-day/ambulatory procedures).

Hospitalization or prolongation of hospitalization in the absence of a precipitating clinical AE is not in itself an SAE. Examples include:

- Admission for treatment of a preexisting condition not associated with the development of a new AE or with a worsening of the preexisting condition (eg, for workup of a persistent pretreatment laboratory abnormality);
- Social admission (eg, subject has no place to sleep);
- Administrative admission (eg, for yearly physical examination);
- Protocol-specified admission during a study (eg, for a procedure required by the study protocol);
- Optional admission not associated with a precipitating clinical AE (eg, for elective cosmetic surgery);
- Hospitalization for observation without a medical AE;
- Preplanned treatments or surgical procedures. These should be noted in the baseline documentation for the entire protocol and/or for the individual subject.

Diagnostic and therapeutic noninvasive and invasive procedures, such as surgery, should not be reported as SAEs. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an SAE. For example, an acute appendicitis that begins during the reporting period should be reported if the SAE requirements are met, and the resulting appendectomy should be recorded as treatment of the AE.

8.3. Severity Assessment

If required on the AE page of the CRF, the investigator will use the adjectives MILD, MODERATE, or SEVERE to describe the maximum intensity of the AE. For purposes of consistency, these intensity grades are defined as follows:	
MILD	Does not interfere with subject's usual function.
MODERATE	Interferes to some extent with subject's usual function.
SEVERE	Interferes significantly with subject's usual function.

Note the distinction between the severity and the seriousness of an AE. A severe event is not necessarily an SAE. For example, a headache may be severe (interferes significantly with the subject's usual function) but would not be classified as serious unless it met one of the criteria for SAEs, listed above.

8.4. Special Situations

8.4.1. Protocol-Specified Serious Adverse Events

Unless the investigator believes that there is a causal relationship between the investigational product and an event specified below, these events should not be reported to Pfizer Safety by the investigator as SAEs. These events are anticipated to occur commonly in a population with DMD. However, these events should still be recorded as AEs on the CRF.

Protocol-specified SAEs that will not normally be reported to Pfizer Safety in an expedited manner:

1. Loss of mobility or ambulation;
2. Muscle weakness;
3. Symptoms related to spinal deformity;
4. Respiratory muscle weakness/Hypoxia;
5. Fracture;
6. Steroid related metabolic changes (eg, hypertension, diabetes).

Should an aggregate analysis indicate that these pre-specified events occur more frequently than expected, eg, based on epidemiological data, literature, or other data, then this will be submitted and reported in accordance with Pfizer's safety reporting requirements. Aggregate analyses of safety data will be performed on a regular basis per internal standard operating procedures.

8.4.2. Potential Cases of Drug-Induced Liver Injury

Humans exposed to a drug who show no sign of liver injury (as determined by elevations in transaminases) are termed "tolerators," while those who show transient liver injury, but adapt are termed "adaptors." In some subjects, transaminase elevations are a harbinger of a more serious potential outcome. These subjects fail to adapt and therefore are "susceptible" to progressive and serious liver injury, commonly referred to as drug-induced liver injury (DILI).

In the majority of DILI cases, elevations in aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) precede total bilirubin (TBili) elevations (>2 × ULN) by several days or weeks. The increase in TBili typically occurs while AST/ALT is/are still elevated above 3 × ULN (ie, AST/ALT and TBili values will be elevated within the same lab sample). In rare instances, by the time TBili elevations are detected, AST/ALT values might have decreased. This occurrence is still regarded as a potential DILI. Therefore, abnormal elevations in either AST OR ALT in addition to TBili that meet the criteria outlined below are considered potential DILI (assessed per Hy's law criteria) cases and should always be considered important medical events, even before all other possible causes of liver injury have been excluded.

The threshold of laboratory abnormalities for a potential DILI case depends on the subject's individual baseline values and underlying conditions. Subjects who present with the following laboratory abnormalities should be evaluated further as potential DILI (Hy's law) cases to definitively determine the etiology of the abnormal laboratory values:

- Subjects with AST/ALT and TBili baseline values within the normal range who subsequently present with AST OR ALT values >3 × ULN AND a TBili value >2 × ULN with no evidence of hemolysis and an alkaline phosphatase value <2 × ULN or not available.

- For subjects with baseline AST **OR** ALT **OR** TBili values above the ULN, the following threshold values are used in the definition mentioned above, as needed, depending on which values are above the ULN at baseline:

    - Preexisting AST or ALT baseline values above the normal range: AST or ALT values >2 times the baseline values AND >3 × ULN; or >8 × ULN (whichever is smaller).

    - Preexisting values of TBili above the normal range: TBili level increased from baseline value by an amount of at least 1 × ULN **or** if the value reaches >3 × ULN (whichever is smaller).

Rises in AST/ALT and TBili separated by more than a few weeks should be assessed individually based on clinical judgment; any case where uncertainty remains as to whether it represents a potential Hy's law case should be reviewed with the sponsor.

In addition, however, as described in Section 1.5.1, in DMD patients, the standard biomarkers of the onset of hepatocellular injury such as ALT and AST are not capable of detection of liver damage due to the release of these enzymes from diseased skeletal muscle. To address the shortcoming, elevations of GLDH >2 x ULN will also be assessed individually based on clinical judgment and with the support of the *ad hoc* hepatic and/or immunopharmacologic expert (s) supporting the E-DMC; any case where uncertainty remains as to whether it represents a potential Hy's law case should be reviewed with the sponsor. Subjects who experience a consistent GLDH elevation above 3 × ULN may also be monitored more frequently to determine if they are an "adaptor" or are "susceptible", as defined above.

If any of the above criteria is met, the subject should return to the investigator site and be evaluated as soon as possible, preferably within 48 hours from awareness of the abnormal results. This evaluation should include laboratory tests, detailed history, and physical assessment.

In addition to repeating measurements of AST and ALT and TBili, laboratory tests should include albumin, creatine kinase (CK), direct and indirect bilirubin, gamma-glutamyl transferase (GGT), GLDH, prothrombin time (PT)/international normalized ratio (INR), total bile acids, alkaline phosphatase and acetaminophen drug and/or protein adduct levels.

Consideration should also be given to drawing a separate tube of clotted blood and an anticoagulated tube of blood for further testing, as needed, for further contemporaneous analyses at the time of the recognized initial abnormalities to determine etiology. A detailed history, including relevant information, such as review of ethanol, acetaminophen (either by itself or as a co-formulated product in prescription or over-the-counter medications), recreational drug, supplement (herbal) use and consumption, family history, sexual history, travel history, history of contact with a jaundiced person, surgery, blood transfusion, history of liver or allergic disease, and potential occupational exposure to chemicals, should be collected. Further testing for acute hepatitis A, B, C, D, and E infection and liver imaging (eg, biliary tract) may be warranted.

All cases demonstrated on repeat testing as meeting the laboratory criteria of AST/ALT and TBili elevation defined above should be considered potential DILI (Hy's law) cases if no other reason for the liver function test (LFT) abnormalities has yet been found. **Such potential DILI (Hy's law) cases are to be reported as SAEs, irrespective of availability of all the results of the investigations performed to determine etiology of the LFT abnormalities.**

A potential DILI (Hy's law) case becomes a confirmed case only after all results of reasonable investigations have been received and have excluded an alternative etiology.

8.4.3. Exposure to the Investigational Product during Pregnancy or Breastfeeding, and Occupational Exposure

Exposure to the investigational product under study during pregnancy or breastfeeding and occupational exposure are reportable to Pfizer Safety within 24 hours of investigator awareness.

8.4.3.1. Exposure during Pregnancy

For both unapproved/unlicensed products and for marketed products, an exposure during pregnancy (EDP) occurs if:

- A female becomes, or is found to be, pregnant either while receiving or having been exposed (eg, because of treatment or environmental exposure) to the investigational product; or the female becomes or is found to be pregnant after discontinuing and/or being exposed to the investigational product:
    - An example of environmental exposure would be a case involving direct contact with a Pfizer product in a pregnant woman (eg, a nurse reports that she is pregnant and has been exposed to chemotherapeutic products).
- A male has been exposed (eg, because of treatment or environmental exposure) to the investigational product prior to or around the time of conception and/or is exposed during his partner's pregnancy.

- A male family member or healthcare provider who has been exposed to the study intervention by skin, mucosal contact, or ingestion then exposes his female partner prior to or around the time of conception.

- A female household member of a study subject reports that she is pregnant and has been exposed through viral shedding by a study subject within 2 months after dosing, and a month prior to or during pregnancy.

If a subject or subject's partner becomes or is found to be pregnant during the subject's treatment with the investigational product, the investigator must report this information to Pfizer Safety on the CT SAE Report Form and an EDP supplemental form, regardless of whether an SAE has occurred. In addition, the investigator must submit information regarding environmental exposure to a Pfizer product in a pregnant woman (eg, a subject reports that she is pregnant and has been exposed to a cytotoxic product by inhalation or spillage) to Pfizer Safety using the EDP supplemental form. This must be done irrespective of whether an AE has occurred and within 24 hours of awareness of the exposure. The information submitted should include the anticipated date of delivery (see below for information related to termination of pregnancy).

Follow-up is conducted to obtain general information on the pregnancy and its outcome for all EDP reports with an unknown outcome. The investigator will follow the pregnancy until completion (or until pregnancy termination) and notify Pfizer Safety of the outcome as a follow-up to the initial EDP supplemental form. In the case of a live birth, the structural integrity of the neonate can be assessed at the time of birth. In the event of a termination, the reason(s) for termination should be specified and, if clinically possible, the structural integrity of the terminated fetus should be assessed by gross visual inspection (unless pre-procedure test findings are conclusive for a congenital anomaly and the findings are reported).

If the outcome of the pregnancy meets the criteria for an SAE (ie, ectopic pregnancy, spontaneous abortion, intrauterine fetal demise, neonatal death, or congenital anomaly [in a live-born baby, a terminated fetus, an intrauterine fetal demise, or a neonatal death]), the investigator should follow the procedures for reporting SAEs.

Additional information about pregnancy outcomes that are reported to Pfizer Safety as SAEs follows:

- Spontaneous abortion includes miscarriage and missed abortion;

- Neonatal deaths that occur within 1 month of birth should be reported, without regard to causality, as SAEs. In addition, infant deaths after 1 month should be reported as SAEs when the investigator assesses the infant death as related or possibly related to exposure to the investigational product.

Additional information regarding the EDP may be requested by the sponsor. Further follow-up of birth outcomes will be handled on a case-by-case basis (eg, follow-up on preterm infants to identify developmental delays). In the case of paternal exposure, the investigator will provide the subject with the Pregnant Partner Release of Information Form

to deliver to his partner. The investigator must document in the source documents that the subject was given the Pregnant Partner Release of Information Form to provide to his partner.

8.4.3.2. Exposure during Breastfeeding

Scenarios of exposure during breastfeeding must be reported, irrespective of the presence of an associated SAE, to Pfizer Safety within 24 hours of the investigator's awareness, using the CT SAE Report Form. An exposure during breastfeeding report is not created when a Pfizer drug specifically approved for use in breastfeeding women (eg, vitamins) is administered in accord with authorized use. However, if the infant experiences an SAE associated with such a drug's administration, the SAE is reported together with the exposure during breastfeeding.

8.4.3.3. Occupational Exposure

An occupational exposure occurs when, during the performance of job duties, a person (whether a healthcare professional or otherwise) gets in unplanned direct contact with the product, which may or may not lead to the occurrence of an AE.

An occupational exposure is reported to Pfizer Safety within 24 hours of the investigator's awareness, using the CT SAE Report Form, regardless of whether there is an associated SAE. Since the information does not pertain to a subject enrolled in the study, the information is not recorded on a CRF; however, a copy of the completed CT SAE Report Form is maintained in the investigator site file.

8.4.4. Medication Errors

Other exposures to the investigational product under study may occur in clinical trial settings, such as medication errors.

Safety Event	Recorded on the CRF	Reported on the CT SAE Report Form to Pfizer Safety Within 24 Hours of Awareness
Medication errors	All (regardless of whether associated with an AE)	Only if associated with an SAE

Medication errors may result from the administration or consumption of the investigational product by the wrong subject, or at the wrong time, or at the wrong dosage strength.

Medication errors include:

- Medication errors involving subject exposure to the investigational product;
- Potential medication errors or uses outside of what is foreseen in the protocol that do or do not involve the participating subject;
- Administration of the incorrect dose level.

Such medication errors occurring to a study subject are to be captured on the medication error page of the CRF, which is a specific version of the AE page.

In the event of a medication dosing error, the sponsor should be notified immediately.

Whether or not the medication error is accompanied by an AE, as determined by the investigator, the medication error is recorded on the medication error page of the CRF and, if applicable, any associated AE(s), serious and non-serious, are recorded on an AE page of the CRF.

Medication errors should be reported to Pfizer Safety within 24 hours on a CT SAE Report Form **only when associated with an SAE**.

9. DATA ANALYSIS/STATISTICAL METHODS

Detailed methodology for summary and statistical analyses of the data collected in this study is outlined here and further detailed in a statistical analysis plan (SAP), which will be maintained by the sponsor. The SAP may modify what is outlined in the protocol where appropriate; however, any major modifications of the primary endpoint definitions or their analyses will also be reflected in a protocol amendment.

9.1. Sample Size Determination

The total sample size of up to 22 subjects across 2 dose cohorts, of 2 sub-populations (ambulatory and non-ambulatory) and the sirolimus cohort is based on clinical considerations to provide adequate safety, tolerability, and pharmacodynamics (PD) information at each dose level for both ambulatory and non-ambulatory subjects.

9.2. Safety Analysis

Adverse events, ECGs, vital signs (blood pressure and heart rate), physical & neurological examinations, cardiac MRI (or echocardiogram) and safety laboratory data (including hematology, chemistry and urinalysis) will be reviewed and summarized on an ongoing basis during the study to evaluate the safety of subjects. Safety data will be presented in tabular and/or graphical format and summarized descriptively, where appropriate. Incidence and severity of TEAEs, withdrawals due to TEAEs, and infusion and injection site reactions will be summarized by dose level and ambulatory status at baseline. Abnormal and clinically relevant changes in physical and neurological examinations, height and weight, vital signs, cardiac MRI (or echocardiogram) and ECG parameters will be summarized by dose level, ambulatory status at baseline, and study day. Incidence and magnitude of abnormal laboratory findings will be summarized by dose level and ambulatory status at baseline.

Medical history and medication history, as applicable, collected during the course of the study will be captured for inclusion into the study database, unless otherwise noted. Any untoward findings identified on physical examination conducted after the administration of the investigational product will be captured as an adverse event, if those findings meet the definition of an adverse event. Data collected at Screening that are used for inclusion/exclusion criteria, such as laboratory data, ECGs and vital signs will be included in the study database. Demographic data collected at Screening will be reported.

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.

9.2.1. Derivation of Vital Signs Parameters

Blood pressures and heart rate will be recorded at each assessment time indicated in the Schedule of Activities. Baseline for vital signs will be defined as the last measurement before the Day 1 investigational product administration.

Vital signs (BP; HR) will be assessed for clinically relevant trends. The standard algorithms and reporting formats will be applied for analyzing all safety data.

Absolute values and changes from baseline for systolic and diastolic blood pressure and heart rate will be summarized by dose level, ambulatory status at baseline and study visit.

Maximum absolute values and maximum changes from baseline for vital signs will also be summarized descriptively by dose level and ambulatory status at baseline using categories as defined in the SAP. Numbers and percentages of subjects meeting the categorical criteria will be provided and individual values listed in the study report.

9.2.2. Electrocardiogram (ECG) Analysis

Baseline for ECG will be defined as the measurement before the Day 1 study drug administration. Changes from baseline for the ECG parameters QT interval, pulse rate, QTcF interval, PR interval and QRS interval will be summarized by dose level, ambulatory status at baseline, and study visit.

The number (%) of subjects with maximum post dose QTc values and maximum increases from baseline in the following categories will be tabulated by dose level and ambulatory status at baseline:

Safety QTcF

	Borderline (msec)	Prolonged (msec)
Absolute Value	≥450 - <480	≥480
Absolute Change	30 - <60	≥60

In addition, the number of subjects with corrected and uncorrected QT values ⩾500 msec will be summarized.

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. 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 subject's individual tracing will be described in a safety section of the study report 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.

CCI [redacted]

9.3. Analysis of Secondary Pharmacodynamic Endpoints

The level of mini-dystrophin expression in muscle biopsies will be assessed by Western blot and/or LC-MS and its distribution by immunofluorescence. Details regarding the analysis will be included in the statistical analysis plan (SAP).

CCI [redacted]

9.5. Interim Analysis

No formal interim analysis (IA) 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. Primary safety analyses and analyses of mini-dystrophin expression and transduction, functional assessments, NAb and ELISpot, viral vector shedding, and health-related outcome measures will occur after the respective study population (ambulatory, non-ambulatory) completes the 1st Year of Follow-up.

9.6. Data Monitoring Committee

The study will utilize an independent E-DMC according to the charter. Members of the E-DMC will not be Pfizer employees, but may include experts in the field of neurology, immunopharmacology, hepatology, complement activation, AAV vectors, and/or statistics. The E-DMC may also include a cardiologist to provide expert interpretation of cardiac MRI (or echocardiogram). A patient advocate will also be included on the E-DMC if an appropriately qualified individual can be identified as agreed upon by the E-DMC chairperson.

The E-DMC will be responsible for ongoing monitoring of the safety as well as benefit/risk profile of subjects 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 subject data as requested by the sponsor 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.

Additional specifics regarding E-DMC responsibilities, inclusive of decisions around dose progression, are detailed in Section 3.5.

10. QUALITY CONTROL AND QUALITY ASSURANCE

Pfizer or its agent will conduct periodic monitoring visits during study conduct to ensure that the protocol and Good Clinical Practices (GCPs) are being followed. The monitors may review source documents to confirm that the data recorded on CRFs are accurate. The investigator and institution will allow Pfizer monitors/auditors or its agents and appropriate regulatory authorities direct access to source documents to perform this verification. This verification may also occur after study completion.

During study conduct and/or after study completion, the investigator site may be subject to review by the IRB/EC, and/or to quality assurance audits performed by Pfizer, or companies working with or on behalf of Pfizer, and/or to inspection by appropriate regulatory authorities.

The investigator(s) will notify Pfizer or its agents immediately of any regulatory inspection notification in relation to the study. Furthermore, the investigator will cooperate with Pfizer or its agents to prepare the investigator site for the inspection and will allow Pfizer or its agent, whenever feasible, to be present during the inspection. The investigator site and investigator will promptly resolve any discrepancies that are identified between the study data and the subject's medical records. The investigator will promptly provide copies of the inspection findings to Pfizer or its agent. Before response submission to the regulatory authorities, the investigator will provide Pfizer or its agents with an opportunity to review and comment on responses to any such findings.

It is important that the investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

11. DATA HANDLING AND RECORD KEEPING

11.1. Case Report Forms/Electronic Data Record

As used in this protocol, the term CRF should be understood to refer to either a paper form or an electronic data record or both, depending on the data collection method used in this study.

A CRF is required and should be completed for each included subject. The completed original CRFs are the sole property of Pfizer and should not be made available in any form to third parties, except for authorized representatives of Pfizer or appropriate regulatory authorities, without written permission from Pfizer.

The investigator has ultimate responsibility for the collection and reporting of all clinical, safety, and laboratory data entered on the CRFs and any other data collection forms (source documents) and ensuring that they are accurate, authentic/original, attributable, complete, consistent, legible, timely (contemporaneous), enduring, and available when required. The CRFs must be signed by the investigator or by an authorized staff member to attest that the data contained on the CRFs are true. Any corrections to entries made in the CRFs or source documents must be dated, initialed, and explained (if necessary) and should not obscure the original entry.

In most cases, the source documents are the hospital or the physician subject chart. In these cases, data collected on the CRFs must match the data in those charts.

In some cases, the CRF may also serve as the source document. In these cases, a document should be available at the investigator site and at Pfizer that clearly identifies those data that will be recorded on the CRF, and for which the CRF will stand as the source document.

11.2. Record Retention

To enable evaluations and/or inspections/audits from regulatory authorities or Pfizer, the investigator agrees to keep records, including the identity of all participating subjects (sufficient information to link records, eg, CRFs and hospital records), all original signed informed consent/assent documents, copies of all CRFs, safety reporting forms, source documents, and detailed records of treatment disposition, and adequate documentation of relevant correspondence (eg, letters, meeting minutes, and telephone call reports). The records should be retained by the investigator according to the ICH guidelines, according to local regulations, or as specified in the clinical study agreement (CSA), whichever is longer.

If the investigator becomes unable for any reason to continue to retain study records for the required period (eg, retirement, relocation), Pfizer should be prospectively notified. The study records must be transferred to a designee acceptable to Pfizer, such as another investigator, another institution, or an independent third party arranged by Pfizer.

Investigator records must be kept for a minimum of 15 years after completion or discontinuation of the study or for longer if required by applicable local regulations.

The investigator must obtain Pfizer's written permission before disposing of any records, even if retention requirements have been met.

12. ETHICS

12.1. Institutional Review Board/Ethics Committee

It is the responsibility of the investigator to have prospective approval of the study protocol, protocol amendments, informed consent/assent documents, and other relevant documents, eg, recruitment advertisements, if applicable, from the IRB/EC. All correspondence with the IRB/EC should be retained in the investigator file. Copies of IRB/EC approvals should be forwarded to Pfizer.

The only circumstance in which an amendment may be initiated prior to IRB/EC approval is where the change is necessary to eliminate apparent immediate hazards to the subjects. In that event, the investigator must notify the IRB/EC and Pfizer in writing immediately after the implementation.

12.2. Ethical Conduct of the Study

The study will be conducted in accordance with the protocol, legal and regulatory requirements and the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences 2002), ICH Guideline for Good Clinical Practice, and the Declaration of Helsinki.

12.3. Subject Information and Consent

All parties will ensure protection of subject personal data and will not include subject names or other identifiable data in any reports, publications, or other disclosures, except where required by law.

When study data are compiled for transfer to Pfizer and other authorized parties, subject names, addresses, and other identifiable data will be replaced by numerical codes based on a numbering system provided by Pfizer in order to de-identify study subjects. The investigator site will maintain a confidential list of subjects who participated in the study, linking each subject’s numerical code to his or her actual identity. In case of data transfer, Pfizer will maintain high standards of confidentiality and protection of subjects’ personal data consistent with applicable privacy laws.

The informed consent/assent documents and any subject recruitment materials must be in compliance with ICH GCP, local regulatory requirements, and legal requirements, including applicable privacy laws.

The informed consent/assent documents used during the informed consent process and any subject recruitment materials must be reviewed and approved by Pfizer, approved by the IRB/EC before use, and available for inspection.

The investigator must ensure that each study subject, or his or her legally acceptable representative, or parent(s) or legal guardian if a minor, is fully informed about the nature and objectives of the study and possible risks associated with participation.

Whenever consent is obtained from a subject's legally acceptable representative/parent(s) or legal guardian, the subject's assent (affirmative agreement) must subsequently be obtained when the subject has the capacity to provide assent, as determined by the IRB/EC. If the investigator determines that a subject's decisional capacity is so limited he/she cannot reasonably be consulted, then, as permitted by the IRB/EC and consistent with local regulatory and legal requirements, the subject's assent may be waived with source documentation of the reason assent was not obtained. If the study subject does not provide his or her own consent, the source documents must record why the subject did not provide consent (eg, minor, decisionally impaired adult), how the investigator determined that the person signing the consent was the subject's legally acceptable representative, the consent signer's relationship to the study subject (eg, parent, spouse), and that the subject's assent was obtained or waived. If assent is obtained verbally, it must be documented in the source documents.

If the study includes minor subjects who reach the age of majority during the study, as recognized under local law, they must re-consent as adults to remain in the study. If the enrollment of emancipated minors is permitted by the study age criteria, the IRB/EC, and local law, they must provide documentation of legal status to give consent without the permission of a parent(s) or legal guardian.

The investigator, or a person designated by the investigator, will obtain written informed consent from each subject or the subject's legally acceptable representative, parent(s) or legal guardian and the subject's assent, when applicable, before any study-specific activity is performed, unless a waiver of informed consent has been granted by an IRB/EC. The investigator will retain the original of each subject's signed consent/assent document.

12.4. Reporting of Safety Issues and Serious Breaches of the Protocol or ICH GCP

In the event of any prohibition or restriction imposed (ie, clinical hold) by an applicable regulatory authority in any area of the world, or if the investigator is aware of any new information that might influence the evaluation of the benefits and risks of the investigational product, Pfizer should be informed immediately.

In addition, the investigator will inform Pfizer immediately of any urgent safety measures taken by the investigator to protect the study subjects against any immediate hazard, and of any serious breaches of this protocol or of ICH GCP that the investigator becomes aware of.

13. DEFINITION OF END OF TRIAL

13.1. End of Trial

The end of trial for the study is defined as the last subject last visit (LSLV).

14. SPONSOR DISCONTINUATION CRITERIA

Premature termination of this study may occur because of a regulatory authority decision, change in opinion of the IRB/EC, or investigational product safety problems, or at the discretion of Pfizer. In addition, Pfizer retains the right to discontinue development of PF-06939926 at any time.

If a study is prematurely terminated, Pfizer will promptly notify the investigators. After notification, the investigators must contact all participating subjects and the site pharmacy (if applicable) within 1 (one) week. As directed by Pfizer, all study materials must be collected and all CRFs completed to the greatest extent possible.

15. PUBLICATION OF STUDY RESULTS

15.1. Communication of Results by Pfizer

Pfizer fulfills its commitment to publicly disclose clinical trial results through posting the results of studies on www.clinicaltrials.gov (ClinicalTrials.gov), the European Clinical Trials Database (EudraCT), and/or www.pfizer.com, and other public registries in accordance with applicable local laws/regulations.

In all cases, study results are reported by Pfizer in an objective, accurate, balanced, and complete manner and are reported regardless of the outcome of the study or the country in which the study was conducted.

www.clinicaltrials.gov

Pfizer posts clinical trial US Basic Results on www.clinicaltrials.gov for Pfizer-sponsored interventional studies (conducted in patients) that evaluate the safety and/or efficacy of a Pfizer product, regardless of the geographical location in which the study is conducted. US Basic Results are submitted for posting within 1 year of the primary completion date (PCD) for studies in adult populations or within 6 months of the PCD for studies in pediatric populations.

PCD is defined as the date that the final subject was examined or received an intervention for the purposes of final collection of data for the primary outcome, whether the clinical study concluded according to the prespecified protocol or was terminated.

EudraCT

Pfizer posts European Union (EU) Basic Results on EudraCT for all Pfizer-sponsored interventional studies that are in scope of EU requirements. EU Basic Results are submitted for posting within 1 year of the PCD for studies in adult populations or within 6 months of the PCD for studies in pediatric populations.

www.pfizer.com

Pfizer posts Public Disclosure Synopses (clinical study report synopses in which any data that could be used to identify individual patients has been removed) on www.pfizer.com for Pfizer-sponsored interventional studies at the same time the US Basic Results document is posted to www.clinicaltrials.gov.

15.2. Publications by Investigators

Pfizer supports the exercise of academic freedom and has no objection to publication by the principal investigator (PI) of the results of the study based on information collected or generated by the PI, whether or not the results are favorable to the Pfizer product. However, to ensure against inadvertent disclosure of confidential information or unprotected inventions, the investigator will provide Pfizer an opportunity to review any proposed publication or other type of disclosure of the results of the study (collectively, "publication") before it is submitted or otherwise disclosed.

The investigator will provide any publication to Pfizer at least 30 days before it is submitted for publication or otherwise disclosed. If any patent action is required to protect intellectual property rights, the investigator agrees to delay the disclosure for a period not to exceed an additional 60 days.

The investigator will, on request, remove any previously undisclosed confidential information before disclosure, except for any study- or Pfizer product-related information necessary to the appropriate scientific presentation or understanding of the study results.

If the study is part of a multicenter study, the investigator agrees that the first publication is to be a joint publication covering all investigator sites, and that any subsequent publications by the principal investigator (PI) will reference that primary publication. However, if a joint manuscript has not been submitted for publication within 12 months of completion or termination of the study at all participating sites, the investigator is free to publish separately, subject to the other requirements of this section.

For all publications relating to the study, the institution will comply with recognized ethical standards concerning publications and authorship, including Section II - "Ethical Considerations in the Conduct and Reporting of Research" of the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, http://www.icmje.org/index.html#authorship, established by the International Committee of Medical Journal Editors.

Publication of study results is also provided for in the CSA between Pfizer and the institution. In this section entitled Publications by Investigators, the defined terms shall have the meanings given to them in the CSA.

If there is any conflict between the CSA and any attachments to it, the terms of the CSA control. If there is any conflict between this protocol and the CSA, this protocol will control as to any issue regarding treatment of study subjects, and the CSA will control as to all other issues.

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Appendix 1. Cohort to Assess the Effect of Sirolimus on Complement Activation Related to PF-06939926 Infusion

Rationale:

An immune response to the viral vector following PF-06939926 infusion has been observed to activate complement and may lead to complement-related SAEs. A hallmark of this immune response is the consistent pattern of decreased platelets and decreased C4 which has been observed within 14 days after infusion with PF-06939926. Sirolimus (Rapamune) prevents B-cell activation[69,70] and has been demonstrated to inhibit anti-AAV9 neutralizing antibody production in non-human primates and to inhibit antibody formation and complement activation after gene therapy in patients with GM1 gangliosidosis (Salabarria et al., 2021 presentation at MDA).

The purpose of this cohort is to evaluate the effect of sirolimus on the measured platelet and C4 levels and NAb antibody titers after infusion of PF-06939926 to determine if sirolimus might serve as an added mitigation strategy to reduce the risk of complement-related SAEs following PF-06939926 infusion.

Dose Rationale:

For subjects weighing ≤40 kg, sirolimus loading dose of 2.25 mg/m^2 BSA twice daily will be administered on Day -10 prior to PF-06939926 infusion, followed by maintenance dose of 0.75 mg/m^2 BSA twice daily. For subjects weighing >40 kg, sirolimus loading dose of 3 mg twice daily will be administered on Day -10 prior to PF-06939926 infusion, followed by twice daily maintenance Dose of 1 mg. The maintenance doses in both the cases will be adjusted to achieve sirolimus trough concentrations within the target range. Please refer to Cohort Intervention table for detailed instructions on the dose, dosing regimen, and maintenance dose adjustments.

The doses were chosen to achieve sirolimus target trough concentration between 4.0 and 7.0 ng/mL in DMD boys. The target levels are lower than those considered for initial treatment of renal transplant patients (pivotal studies included levels >10 ng/mL for the first year, and now with more typical target ranges of 6-12 ng/mL during the first 6 months), as the goal of sirolimus dose in this study is to suppress the humoral immune response in the initial post-treatment period when the AAV viral vector levels are high. To determine a lower, effective dose, a non-clinical study in cynomolugus monkeys was performed. In this study, following administration of AAV9, low dose sirolimus trough levels (<3 ng/mL) were ineffective in suppressing the rise of NAb, whereas higher dose trough levels (~3-4 ng/mL) were effective. Given the variability among subjects' trough levels at a given dose, the target trough range was extended up to 7 ng/mL to ensure adequate immune suppression while also minimizing the risk for adverse events.

Population PK approaches were considered to guide selection of the proposed dose and dose regimen. The weight and height distributions for the simulations were taken from growth charts specific to DMD boys[72], as well as the Cooperative International Neuromuscular

Research Group (CINRG) dataset. Sirolimus concentrations in DMD boys were simulated using a Population PK model which included both pediatric and adult populations.[73] The model predicted that a loading dose of 2.25 mg/m^2 BSA twice daily followed by maintenance dose of 0.75 mg/m^2 BSA twice daily for subjects ≤40 kg, and a fixed loading dose of 3 mg twice daily followed by maintenance doses of 1 mg twice daily for subjects >40 kg will achieve target trough concentration between 4.0 and 7.0 ng/mL in majority of the subjects.

Objectives and Endpoints:

Objective:	**Endpoints:**
To assess the effect of sirolimus on complement activation when administered according to the protocol.	• Percent change from baseline of nadir in C4 levels through Day 30. • Percent change from baseline of nadir in platelet levels through Day 30. • Change from baseline of NAb antibody titers through Day 30.
To evaluate the safety of sirolimus when administered according to the protocol.	• Incidence, severity, and causal relationship of treatment-emergent AEs through 30 days post-treatment. • Incidence and magnitude of abnormal laboratory findings through 30 days post-treatment.

Cohort Design:

For this cohort, in addition to all study procedures and timing in the Study C3391001 protocol, subjects will receive sirolimus 10 days prior to and for 14 days following infusion with PF-06939926.

Information for sirolimus may be found in the Single Reference Safety Document, which for this study will be the United States Prescribing Information.[71] Additional safety information on potential risk related to the use of sirolimus in the pediatric population is provided below.

Cohort Schedule of Activities:

All procedures detailed in the protocol will be conducted as detailed in the Schedule of Activities. Additionally, for this cohort, the following will apply:

Period				**Treatment**				
Visit Number	**Visit 2.2[a, d]**	**Visit 2.3**	**Remote Visit 2.4 Phone Contact**	**Visit 3**	**Remote Visit 3.1 Phone Contact**	**Visit 4 Week 2**	**Remote[d] Visit 4.2**	**Remote[d] Visit 5.2**
Visit Window	**Day -10**	**Day -7**	**Day -5[b] (+3 days)**	**Day 4 (±1 day)**	**Day 6 [b, c] (+3 days)**	**Day 14 (±1 day)**	**Day 17 (±1 day)**	**Day 25 (±1 day)**
Administration of sirolimus	X	→	→	→	→	X		
Platelets and C4 Levels							X	X
Sirolimus trough level (locally)		X		X		X		
Sirolimus dose level adjustment			X		X			

a. Phone communication in the AM between site staff and subject and/or caregiver as a reminder to begin taking the sirolimus if dispensed previously at the baseline visit.
b. Sirolimus dose adjustments once the trough concentration level from Day -7 is available but at least 2 days prior to dosing.
c. Sirolimus dose adjustments once the trough concentration level from Day 4 is available.
d. Unless clinical concern and/or subject preference warrants an in-person visit, remote visits may be performed.

Number of Subjects:

Up to 8 non-ambulatory subjects will receive a single dose of PF-06939926 in this cohort. Additionally, for the subjects in this cohort, sirolimus will be administered as described in the table below. In order to mitigate unanticipated risks to subject safety, the PF-06939926 dosing interval will be at least 3 weeks between subjects for the first 3 subjects. If no safety concerns are identified 3 weeks after the third subject is infused, then PF-06939926 dosing may proceed at ≥2 week intervals for the additional subjects in the cohort.

Pending review of data in non-ambulatory subjects, up to 5 ambulatory subjects may also be included in this cohort, as determined by the sponsor. Continued dosing at any point in this cohort will be at the discretion of the sponsor, based on emerging data.

Subject Eligibility Criteria:

The inclusion and exclusion criteria below is in addition to meeting all eligibility criteria listed in Section 4. The inclusion criterion of age described here will supersede that in Section 4.

Inclusion Criteria

Subjects must meet the following inclusion criterion to be eligible for enrollment into the sirolimus cohort:

1) >8 years of age

Exclusion Criteria

Subjects are excluded from this cohort if any of the following criteria apply:

1) Hypersensitivity to sirolimus or intolerance to soy, including a history of angioedema;

2) Concomitant use with strong CYP3A4/P-gp inducers (including but not limited to rifampin, rifabutin, or St. John's wort) or strong CYP3A4/P-gp inhibitors (including but not limited to ketoconazole, voriconazole, itraconazole, erythromycin, telithromycin, or clarithromycin) that decrease or increase sirolimus concentrations. The full list may be found at the US Food & Drug Administration website. (Drug Development and Drug Interactions: Table of Substrates, Inhibitors and Inducers.)

Potential risks associated with experimental use of sirolimus in pediatric subjects:

Additionally to potential risks listed in the sirolimus label[71] for the 2 indicated populations, a targeted review of post-marketing off label use reports from pediatric subjects within 30 days of starting sirolimus was performed and identified the following additional potential risks:

Based on a cumulative safety review (through 31 March 2021) of 255 cases related to the off label pediatric use within 30 days of starting sirolimus administration, the observed safety profile did not significantly differ from the one described in the label for the approved indications.[71] The majority of reported SAEs were reported in the system organ classes (SOCs) of Blood and lymphatic system disorders, Infections & Infestations, and Respiratory, thoracic and mediastinal disorders. The anticipated potential risks associated with short term use of sirolimus in the pediatric DMD population may include:

- mouth ulcer (stomatis, mouth ulceration, mucosal disorder, oral mucosal blistering);

- gastrointestinal disorders (nausea, diarrhaea, vomiting, haematemesis, abdominal pain/discomfort);
- metabolic disorders (hyperlipidaemia, hypertriglyceridaemia, dyslipidaemia);
- skin conditions (skin rash, skin erosion, skin irritation);
- increased susceptibility to infection (label warning: herpes zoster, bacterial pneumonia, sepsis, septic shock, cellulitis);
- liver abnormalities (hepatic function abnormal, increased ALT and/or AST);
- impaired healing (also in Warnings and Precautions);
- pyrexia;
- swelling/edema.

As per the warnings in the sirolimus label, there is a potential risk of angioedema with sirolimus alone, and with concomitant use of ACE inhibitors. If angioedema were to occur in this study during treatment with sirolimus, sirolimus dosing should be discontinued immediately. Subjects with angioedema prior to infusion with PF-06939926 will be withdrawn from this cohort. The following AEs may be challenging to evaluate for relatedness if they occur during the co-administration period, since they have been reported for both sirolimus and PF-06939926 during the first 2 weeks of administration: hypertension, anemia, nausea, diarrhea, headache, fever, thrombocytopenia, hemolytic uremic syndrome, thrombotic microangiopathy.

Cohort Intervention: Subjects in this cohort will receive oral sirolimus solution as follows:

Subject Age/ Weight Range	**≤40.0 kg**	**>40.0 kg**
Day -10 before PF-06939926 infusion	Oral sirolimus solution at loading dose of 2.25 mg/m^2 BSA given twice daily.	Oral sirolimus solution at loading dose 3 mg given twice daily.
Day -9 to Day -1 (or sooner if dose adjustment is required based on the Day -7 trough concentration level) before PF-06939926 infusion and on the morning of Day 1.	Oral sirolimus solution at maintenance dose of 0.75 mg/m^2 BSA given twice daily. **Day -7 before PF-06939926 infusion:** Draw blood to measure sirolimus trough concentration (*local lab*).	Oral sirolimus solution at maintenance dose of 1 mg given twice daily. **Day -7 before PF-06939926 infusion:** Draw blood to measure sirolimus trough concentration (*local lab*).

Subject Age/ Weight Range	**≤40.0 kg**	**>40.0 kg**
Based on the Day -7 sirolimus trough concentration, adjust the dose on the day that the trough concentration level is received, if needed, to achieve target level of 4-7 ng/mL	**If trough concentration is <3.9 ng/mL**, increase dose by the following formula: current dose × (5.5 ng/mL ÷ current concentration).	**If trough concentration is <3.9 ng/mL**, increase dose by the following formula: current dose × (5.5 ng/mL ÷ current concentration).
	If trough concentration is within target range (4.0-7.0 ng/mL), keep at 0.75 mg/m^2 BSA twice a day.	**If trough concentration is within target range (4.0-7.0 ng/mL)**, keep at 1 mg twice a day.
	If trough concentration is 7.1-14.0 ng/mL, reduce dose using the following formula: current dose × (5.5 ng/mL ÷ current concentration).	**If trough concentration is 7.1-14.0 ng/mL**, reduce dose by the following formula: current dose × (5.5 ng/mL ÷ current concentration).
	If trough concentration is >14.0, skip 1 dose and reduce dose by the following formula: current dose × (5.5 ng/mL ÷ current concentration).	**If trough concentration is ≥14.0**, skip 1 dose and reduce dose by the following formula: current dose × (5.5 ng/mL ÷ current concentration).
Days 1-14 post-PF-06939926 infusion	Oral sirolimus solution at maintenance dose as determined above using trough concentrations.	Oral sirolimus solution at maintenance dose as determined above using trough concentrations.
Day 4 (±1 day) post-PF-06939926 infusion	Draw blood for trough concentration for possible readjustment (same as described above).	
Day 14 post-PF-06939926 infusion	Draw blood for trough concentration to assess levels.	

For subjects who weigh ≤ 40 kg, the required dose of sirolimus is dependent of the subject's body surface area (BSA). BSA can be calculated per below guidance[74].

$$BSA\ (m^2) = \sqrt{\frac{Ht\ (cm) x\ Wt\ (kg)}{3600}}$$

The maximum sirolimus dose administered on any day should not exceed 40 mg. The duration of sirolimus administration can be extended, if needed, based on laboratory data consistent with an ongoing immune reaction, only after consultation and if agreed by the sponsor.

Additional guidance while providing sirolimus is listed below:

- Investigators should review the product label information[71] and the information provided above (see potential risks associated with experimental use of sirolimus in pediatric subject).

- Investigators should discuss any modifications to dosing regimen with the sponsor if a subject begins taking medications known/listed to interact with CYP3A4 or P-gp metabolism.

- Investigators should advise subjects to refrain from consuming grapefruit juice while taking sirolimus.

- Investigators should advise subjects that, to minimize variability in sirolimus concentrations, sirolimus should be taken either consistently with food or consistently without food.

Study Treatments:

Subjects will temporarily replace their daily glucocorticoid regimen as described in Section 5. Pending review of the data, at the discretion of the sponsor, this regimen may be changed to:

1. Between 1 and 4 hours prior to infusion of PF-06939926: receipt of single dose of IV methylprednisolone ⩾1 mg/kg;

18. For Weeks 1, 2, 3, and 4 post-infusion of PF-06939926: switch of daily glucocorticoid dose to ⩾1 mg/kg/day of oral prednisone or prednisolone;

19. For Weeks 5, 6, 7, and 8 post-infusion: reduction of oral prednisone or prednisolone dose to a minimum of 0.75 mg/kg/day;

20. For Weeks 9, 10, 11, and 12 post-infusion: reduction of oral prednisone or prednisolone dose to a minimum of 0.5 mg/kg/day;

21. After Week 12 post-infusion: return to pre-study daily doses of prednisone or prednisolone.

CCI [redacted]

[redacted]

[redacted]

[redacted]	[redacted]

CCI

Appendix 2. Alternative Measures During Public Emergencies

The alternative study measures described in this section are to be followed during public emergencies, including the COVID-19 pandemic. This appendix applies for the duration of the COVID-19 pandemic globally or specific locations(s) and will become effective for other public emergencies only upon written notification from Pfizer.

Use of these alternative study measures are expected to cease upon the return of business as usual circumstances (including the lifting of any quarantines and travel bans/advisories).

Telehealth Visits

In the event that in-clinic study visits cannot be conducted, every effort should be made to follow-up on the safety of study subjects at scheduled visits per the Schedule of Activities or unscheduled visits. Telehealth visits may be used, if/when it is not possible to have home visits conducted by a home healthcare service (see **Home Health Visits**), to continue to assess subject safety and collect data points. Telehealth includes the exchange of healthcare information and services via telecommunication technologies (eg, audio, video, video-conferencing software) remotely, allowing the subject and the investigator to communicate on aspects of clinical care, including medical advice, reminders, education, and safety monitoring. The following assessments must be performed during a telehealth visit:

- Review and record any AEs and SAEs since the last contact. Refer to Section 8.
- Review and record any new concomitant medications or changes in concomitant medications since the last contact.
- Confirm that the subject is adhering to the contraception method(s) required in the protocol. Refer to Section 4.5.2.

Study subjects must be reminded to promptly notify site staff about any change in their health status.

Alternative Facilities for Safety Assessments

Laboratory Testing

If a study subject is unable to visit the site for protocol-specified safety laboratory evaluations, testing may be conducted at a local laboratory if permitted by local regulations. The local laboratory may be a standalone institution or within a hospital. The following safety laboratory evaluations may be performed at a local laboratory (see Table 1):

- Hematology panel
- Chemistry panel
- Urinalysis

- Other (as defined in Table 1).

If a local laboratory is used, qualified study site personnel must order, receive, and review results. Site staff must collect the local laboratory reference ranges and certifications/accreditations for filing at the site. Laboratory test results are to be provided to the site staff as soon as possible. The local laboratory reports should be filed in the subject's source documents/medical records. Relevant data from the local laboratory report should be recorded on the CRF.

Home Health Visits

A home health care service will be utilized to facilitate scheduled visits per the Schedule of Activities. Home health visits include a healthcare provider conducting an in-person study visit at the subject's location, rather than an in-person study visit at the site. The following may be performed during a home health visit:

- Physical exam
- Blood draws
- Urine sample collection
- Vital signs
- C-SSRS
- [redacted]
- [redacted]
- [redacted]
- Clinical outcome assessments.

Appendix 3. Protocol Amendment History

The Protocol Amendment Summary of Changes Table for the current amendment is located directly before the table of contents (TOC). The protocol amendment summary of changes tables for past amendment(s) can be found below:

Original Protocol	20 July 2017	Not applicable (N/A)
Amendment 1	03 December 2018	CCI [redacted] 1. Addition of 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 highest dose level; CCI [redacted] 3. Allowance for the option of broadening the window between the Baseline and Dosing visits to accommodate scheduling challenges and ensure timely delivery of PF-06939926 to site for dosing; 4. Allowance for more flexibility as far as the method for collecting muscle biopsies (ie, open and/or needle); 5. Allowance for the option of performing a locally-read echocardiogram if attempted cardiac MRI cannot be evaluated and a repeat is unlikely to resolve the issue; 6. Addition of oral prednisolone as an option for the 3 months following study drug administration;

		7. Allowance to modify the regimen of the required background glucocorticoid regimen after the first year post-treatment; 8. Removal of language that inadvertently indicated that 12 planned subjects would be split evenly between the 2 planned cohorts; 9. Broadening of the option of adding several additional subjects to either cohort for reasons other than safety (eg, to also better understand any preliminary efficacy data); 10. Clarification that a pause to enrollment for assessment of the biopsies obtained from first 3 subjects' of the second cohort would only be necessary if no measurable transgene expression was observed in the first cohort (ie, lower dose level); 11. Clarification that receipt of the influenza vaccine within 30 days prior to receipt of study drug or 3 months after is prohibited; 12. Allowance for flexibility of the weight-based calculations for the required glucocorticoids for practical purposes and to align with standard of care; 13. Emphasis that 2 site personnel should confirm subject information entered into the electronic system for enrollment, as these data are critical for ensuring that the appropriate amount of PF-06939926 is shipped to the site for the given subject; 14. Clarification that infusion site monitoring is only required from start of infusion through second month post-infusion, unless clinically indicated; 15. Clarified that any cardiac MRI taken within 3 months prior to Screening and deemed adequate for confirming eligibility for this study may replace the need for a Screening MRI;

		16. Replaced “study entry” with “the Baseline visit” for clarity; 17. Administrative clarifications (eg, to ensure consistency between Schedule of Activities and other sections of the protocol).
Amendment 2	14-Feb-2019	1. In response to observed nausea, vomiting and symptoms resembling acute viral gastroenteritis following the administration of PF-06939926 in this current trial, additional safety monitoring has been incorporated into the Treatment period (ie, if adverse events [AEs] possibly related to PF-06939926 are observed, subjects should not be discharged until the events have resolved or can be managed by the subject and/or caregiver; upon discharge, subjects should stay near the investigational site to enable easy follow-up in the event of any emergent AEs through the Week 1 visit, or longer if clinically indicated; subjects may also return to the investigational site on Day 4 [±1 day] for safety and viral vector shedding biospecimen collection, as well as assessment of any new or worsening adverse events or changes to concomitant medications); 1) Guidance added for practicing good general hygiene for 1-2 months following administration of PF-06939926 in the Activity section of the protocol (Section 4.5.1), given that PF-06939926 is of a viral vector; 2) To obtain better characterization of the viral vector shedding kinetics during the first year following administration of PF-06939926, 4 more biospecimen collection timepoints have been added. Safety will also be monitored at these additional visits via collection of safety labs and discussion with the subject regarding any new or worsening adverse events or changes in concomitant medications;

		3) Extension of the Screening visit window from 84 to 126 days prior to Day 1 (or 112 days relative to Baseline) to allow for more flexibility in the event of enrollment pauses. However, should the period between Screening and Baseline visits exceed 84 days (12 weeks) for a given subject, then the Screening labs, thigh and upper arm imaging, and functional assessments should be repeated to (re)confirm eligibility prior to enrollment; 4) Mention that medications may be given to treat or prevent adverse events possibly related to PF-06939926 (eg, antiemetics or antihistamines for nausea and vomiting events) (Section 5.7.1); 5) Administrative changes to improve consistency and clarity throughout document (eg, correction to the study design schematic in which the long-term follow-up for Cohort 2 was accidentally omitted; correction in duration of study from 4 to 5 years of follow-up; clarification in Section 4.2 that cardiac magnetic resonance imaging from within 3 months prior to Screening can be used to assess eligibility as already described in Section 6.1).
Amendment 3	10 May 2019	1. In response to observed acute renal injury associated with complement activation following the administration of PF-06939926, additional safety monitoring and appropriate medical management has been incorporated into the Treatment and 1st Year Follow-up periods: • If adverse events (AEs) indicative of renal injury and/or complement activation occur, subjects will be evaluated promptly by the investigator and/or medically qualified individual(s) for proper care and management; • Upon discharge from the planned 24-hour stay, subjects should stay near the

		investigational site to enable easy follow-up in the event of any emergent AEs through the Day 14 (Week 2) visit, which will now be in-clinic, or longer if clinically indicated; • Subjects will return to the investigational site on Day 10 (in addition to Days 4, 7 and 14) for biospecimen collection, as well as for assessment of any new or worsening adverse events, changes to concomitant medications, and confirmation of adequate fluid intake and output; • Addition of a remote Day 21 (Week 3) biospecimen collection and wellness check-in visit; • Addition of triplicate electrocardiograms at Weeks 2 and 4; • Addition of body temperature collected at all timepoints for vitals from prior to PF-06939926 infusion through Week 2, and again at Week 4; [redacted] • Requirement for eculizumab to be accessible by the investigational sites for use if complement activation observed; • Guidance that eculizumab use would require either prior meningococcal vaccination (at least 2 weeks prior to dosing) and/or prophylactic antibiotics for

		meningococcus for the duration eculizumab is needed; • Guidance added for monitoring fluid intake and urine output through Week 4 following administration of PF-06939926 in the Activity section of the protocol (Section 4.5.1). 1) Shift of several safety assessments (ie, Physical and Neurological Exam, Columbia Suicide Severity Rating Scale) from Day 7 to Day 14, now that Day 14 is an in-clinic (previously a remote visit). 2) Additional extension of the Screening visit window from 126 to 196 days prior to Day 1 (or 182 days relative to Baseline) to allow for more flexibility in the event of enrollment delays or pauses so as to minimize burden on subjects who would otherwise be required to unnecessarily repeat all Screening assessments. However, as is reflected in Amendment 2, should the period between Screening and Baseline visits exceed 84 days (12 weeks) for a given subject, then the Screening labs, thigh and upper arm imaging, and functional assessments should be repeated to (re)confirm eligibility prior to enrollment. 3) Allowance for flexibility in timing of post-treatment muscle biopsies (ie at either 2 or 3 months and either 6 or 12 months vs. only at 2 and 12 months) for better characterization of peak transgene expression and distribution. The specific timepoint for each biopsy for a given subject will be determined by the sponsor but with investigator input. Despite this newly added flexibility, no subject will undergo muscle biopsies at more than 3 total visits. 4) Modification to lower limit of age range for eligibility from 5 years old to 4 years old, so as to acquire experience and safety data in the

		full age range planned for the next phase of clinical drug development. 5) Allowance for reduced pause between subjects, with external data monitoring committee (E-DMC) approval, provided that 6 consecutive subjects who are dosed at the current 3-6 week intervals at the given dose level meet no enrollment pause criteria. 6) Allowance for use of prophylactic antiemetics to assess whether these are effective in preventing nausea and/or vomiting during the first 7-10 days following administration of PF-06939926. 7) Update of blood sampling limits in this pediatric study, given the recent safety events that require additional safety monitoring and blood tests, and particularly for this type of gene therapy study with a single infusion, the first several months after which are most critical. 8) Removal of erythrocyte sedimentation rate (ESR), given that this local laboratory test does not appear to be a useful marker for identifying inflammatory events that may be related to PF-06939926, and even less so given the additional lab tests and safety monitoring included under this amendment (as described above). 9) Administrative changes to improve consistency and clarity throughout document (eg, fixed typographical error in study drug name within study schematic; clarification that enrollment pause criteria due to safety applies to any dose level; clarified that safety risk identified during this study will be described in the Investigator's Brochure).
Amendment 4	20 February 2020	1. Number of subjects to be enrolled increased from 12 to 24 in an effort to better evaluate

		safety of PF-06939926, per availability of drug product. CCI [redacted] 2) Increased the duration of length of entire study given increase in number of subjects to be enrolled, which is helpful for investigators to set expectations as to when they can expect final study results, and increased duration of subject participation from approximately 5 years to 5.5 years, given the extended Screening window as per Amendment 2. 3) Addition of possible local laboratory samples to be collected and tested on Study Days 7-10 and extension of complement biomarker collection through Week 8, or perhaps longer if outside normal range, so as to ensure better understanding of possible changes in laboratory results during this period, as well as to allow for closer safety monitoring per communications between the investigators and sponsor. 4) Removed reference to “blood smear” in Schedule of Activities, Section 6.3-6.6 and Table 1, given that this not a separate test and is already part of the hematology panel since the start of the study. 5) Assessment of drug concentrations of PF-06939926 changed to a titer method, which will be used to adjust referenced doses. Use of the more precise titer assay was implemented per regulatory request. 6) Broadened flexibility to increase the dosages of the protocol required glucocorticoids just prior to (ie, methylprednisolone) and for at

		least 3-months following administration of PF-06939926 to explore whether increased doses may mitigate risk of adverse immune reactions. 7) Expanded the list of examples of medications that can be administered to treat or prevent adverse events to include immunosuppressants and complement inhibitors as these may be used to mitigate risk of complement activation. 8) Removed reference to a 6 milliliter (mL) vial of investigational product, PF-06939926, as the vial size will be increased; the fill volume will remain 5 mL, as reflected. CCI 
Amendment 5	20 July 2020	1. Expanded study population to include non-ambulatory subjects with Duchenne muscular dystrophy (DMD), in an effort to broaden the safety evaluation of PF-06939926 in this patient population (Title page, Section 3.1, and as noted below),

		1) Updated current therapies for DMD given recent regulatory approval of another exon-skipping medication, ie, VYONDYS 53™ (Section 1.2.1). 2) Broadened Rationale for Selected Patient Population Section (1.4.3) to include non-ambulatory subjects. 3) Removed flexibility around biospecimen collections for local laboratory testingfollowing administration of PF-06939926, such that local laboratory testing is now required on Days 5-9 to ensure consistent safety monitoring during this period (Schedule of Activities, Sections 3.3, 6.3). CCI  5) Increased number of subjects to be enrolled from 24 to up to approximately 30 to accommodate up to approximately 20 ambulatory DMD subjects, and now up to approximately 10 non-ambulatory DMD subjects, per availability of drug product (Sections 1.4, 3.1, 3.2, 9.1 Figure 2, and throughout protocol). 6) Added flexibility to broaden duration of infusion and total volume of PF-06939926, as these may be needed for subjects >50 kg (Sections 3.1, 5.4). 7) Removed the anti-smooth muscle antibody assay and anti-liver kidney microsomal antibody type 1 laboratory tests for autoimmune-mediated hepatitis as Exclusion

		Criteria, given the lack of observed hepatic injury associated with PF-06939926 (Schedule of Activities, Sections 4.2, 7.2.1, Abbreviations). 8) Increased the dose of intravenous methylprednisolone prior to dosing from ⩾ 1 to ⩾2 mg/kg, and similarly for oral prednisone or prednisolone from ⩾ 1 mg/kg/day to ⩾2 mg/kg/day for the first 2 weeks following PF-06939926 in order to further mitigate risk of an adverse immune response, before adjusting back down to ⩾ 1 mg/kg/day for the remainder of the first month (Schedule of Activities, Section 5, 5.4, 6.3, 6.4). 9) Included mention of other inactivated vaccinations beyond influenza by which administration would need to be restricted relative to administration of PF-06939926, in order to accommodate possible forthcoming vaccinations, such as that for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Sections 4.2 and 5.7.2). 10) Added new and clarifying language to accommodate addition of non-ambulatory subjects in the study including the following: a. Clarified that consent will be obtained directly from subjects of age of majority, and differentiated role of caregivers for subjects who are minors (Sections 4, 6.1); b. Modified certain eligibility criteria to accommodate non-ambulatory subjects (ie, age, weight, functional performance, cardiac function) (Section 4); c. Added distinction between functional assessments required for ambulatory

		vs. non-ambulatory subjects (Schedule of Activities, Sections 6, 7.5); d. Added flexibility in the neurological examination for non-ambulatory subjects, since certain domains (eg, station and gait) will not apply to this sub-population (Section 7.2.3); e. Included instructions for measuring height and weight of non-ambulatory subjects (Section 7.2.4, referenced in Section 5.3.1); f. Removed requirement that subjects are to be supine for vital signs collection in order to eliminate an unnecessary burden on non -ambulatory subjects (Section 7.2.5 and throughout); g. Added language around managing changes in cardiac imaging for non-ambulatory subjects (Section 7.2.7.2); h. Added adult versions of the Columbia Suicide Severity Rating Scale (C-SSRS) to be completed directly with subjects who are >12 years of age at Screening, and added language that site staff can physically complete the form on behalf of the subject, if needed (Section 7.2.8, as well as Schedule of Activities, Section 6); CCI [redacted] j. Added flexible language around the collection of biopsies for both ambulatory (as per the Protocol Administrative Change Letter, dated 03Oct2019) and now specifies the muscle groups for collection of needle

		biopsies, as well as details regarding collections from non-ambulatory subjects (Section 7.6, and 1.4.2, which indicates that up to a maximum of 3 biopsy procedures will occur per subject); k. Added new Clinical Outcomes Assessments for the DMD non-ambulatory sub-population to be included with this amendment and added clarification around which assessments are to be completed by which subjects based on ambulatory status and age (Section 7.7, as well as Sections 2 and 6); l. Added clarification that data analyses may also be split by ambulatory status (Section 9.4). 11) Removed specification that echocardiograms should be performed using a 2-dimensional (2-D) imaging collection method, as many sites may now have 3-dimensional (3D) ejection fraction capabilities (Section 7.2.7.2). 12) Added clarification around documentation of medication compliance if the subject dosing diary for daily oral prednisone or prednisolone is not utilized (Schedule of Activities, Section 7.2.9.1). 13) Clarified that version 2.0 of the Performance of Upper Limb scale is used in this study (Schedule of Activities, Sections 4.1, 6.1, 7.5.6). 14) Added clarification that ideally the same caregiver will complete the clinical outcomes assessments throughout the study for consistent reporting, when feasible (Section 7.7). 15) Removed detail around expected division of subjects between cohorts and dose escalation

		from Sample Size Determination (Section 9.1), because already described elsewhere (Sections 3.1, 3.2 and Section 3.5, respectively). 16) Included other areas of expertise that may be incorporated into the E-DMC (Section 9.6). 17) Administrative changes to improve consistency and clarity throughout document (eg, updated appendix of abbreviations to include new terms; replaced previously defined terms with abbreviations or acronyms where appropriate; corrected error for hybrid, not human, creatine kinase [hCK] [Section 1.1]: corrected error to reflect Zarit Burden Interview vs. Zarit Burdent Inventory [Section 7.1.1.1 and Abbreviations]; added clarity for consistency that daily glucocorticoid regimen is required and compliance should be captured through at least 1 year after receipt of PF-06939926 [Schedule of Activities, Sections 1.5.1, 5]; added clarity around permitted medications to treat or prevent adverse events [Section 5.7.1]; adjusted placement of clarification for repeat Screening labs [Section 6.1]); added clarification that concomitant medications or therapies may also be considered when assessing the causality of a serious adverse event.
Amendment 6	03 March 2021	1) Added the following Exclusion Criteria: • For clarification: Prior treatment with gene therapy, defined as any therapy introducing exogenous DNA or intended to permanently alter the endogenous DNA. Gene therapy (other than study drug) will be prohibited for the duration of the study. • For clarification: Exposure within 6 months prior to Screening (Visit 1) to any treatment designed to increase

		dystrophin expression (including, but not limited to exon-skipping agents and nonsense read-through). These treatments will also be prohibited during the period between Screening (Visit 1) and Day 1 (Visit 3) and for the first year of the study. • To prevent hypersensitivity reactions: Known hypersensitivity to any of the components of the study drug or solution for infusion, such as hypersensitivity to albumin or a diagnosis of (or symptoms suggestive of) hereditary fructose intolerance (HFI). Symptoms suggestive of HFI include nausea, vomiting, bloating, stomach cramps, or diarrhea following the ingestion of sweet foods or drinks, or a pattern of avoiding sweet foods or drinks. • To account for elevated values as a result of treatment with steroids that do not reflect renal impairment: Changed Cystatin C criterion from > 1.0 mg/L to >1.2 × ULN • Platelets <150 × 10^3/μL; 2) For clarification, added the length of time that the study drug is stable. (Section 5.4) 3) Schedule of Activites - Added that the baseline NAb to AAV: will be collected and sent to the central laboratory, but will only be analyzed and reviewed prior to Day 1 (Visit 3), if the study team determines that Day 1 (Visit 3) will be more than 55 days after the Screening visit (Visit 1). 4) Clarified the use of cardiovascular medications and changed may to must for the receipt of the Meningococcal Vaccine.

		(Section 5.7.1) so that there is consistency with among all protocols for this program. 5) Added Cardiac Troponin to Triggers for Additional Safety Monitoring (Section 7.2.12). 6) Clarified when the primary safety analysis will be conducted (Section 9.5). 7) To allow for the sites to have flexibility, added that trained respiratory therapists can perform PFTs (Section 7.5). 8) Added an appendix to include alternative measures during public emergencies (Appendix 1). 9) Added additional abbreviations to Appendix 2. CCI [redacted]
Amendment 7	03 June 2021	1) Section 1.5.3 Other Risks: Added the possible risks of the use of a PICC line for blood draws since a PICC line may be used. 2) Section 1.4 Study Design Rationale, Section 3.1 Study Overview, Section 3.2 Planned Number of Subjects, and Section 9.1 Sample Size Determination: changed the sample size to 35. 3) Section 3.5 Dose Progression and Stopping Rules: 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 subjects with the clinical events that prompted the review instead of all available data. The E-DMC has reviewed 3 cases of elevated GLDH levels and agreed that the review of subsequent cases was not necessary unless Hy's Law was met.

		4) Section 5 Study Treatments: Adjusted the glucocorticoid regimen post-infusion with PF-06939926 to allow for a slower tapering and to minimize the risk of adrenal insufficiency. 5) Section 5.7.2 Prohibited Therapies: Added that subjects will be able to receive an mRNA, or DNA-based, or non-replicating viral vector vaccine (eg, against SARS-CoV2) within specified time windows. 6) Added a requirement that subjects who experience an event compatible with aHUS will be screened for a genetic predisposition to aHUS to obtain information that may contribute to an understanding of the risk for developing aHUS. 7) Section 7.2.1 Clinical Laboratory: Clarified that the PICC line can only be used for blood draws and not study drug administration. 8) Table 1 Central Laboratory Tests: Added magnesium to the chemistry panel. 9) A cohort was added as an appendix to evaluate the use of sirolimus to mitigate the risk of complement-mediated events related to PF-06939926. 10) Changed “first” cohort to “low-dose” cohort and “second” cohort to “high-dose” cohort throughout the document.
Amendment 8	02 September 2021	1) Schedule of Activities Footnote I and Section 6.1: Changed “may’ to “will” be collected and tested for the ADA to mini-dystrophin at screening and added ADA to mini-dystrophin at Week 2/Day 14 to the SoA and Section 6.4. This change is to identify subjects with a potentially higher risk of immune reactions. 2) Schedule of Activities Footnote L: added that DMD genetic testing will only be

		obtained and sent to the central lab if required to confirm the diagnosis of DMD. 3) Section 4.2 Exclusion criteria: Addition of exclusion criterion to exclude subjects with specific mutations in the dystrophin gene considered to increase the risk of immune-mediated adverse reactions. This was a recommendation by the E-DMC based on the review of the immune and genetic data from studies C3391001 and C3391003. 4) Appendix 1: Sirolimus Cohort Schedule of Activities: Remote Visit 2.4 Phone Contact, the range of the visit window was changed from "+2 days" to "+3 days" to accommodate for the length of time it could take to receive the sirolimus trough concentration level back from the lab. Also removed the footnote that referenced the Platelet and C4 levels and changed "additional clinical safety labs" to "Platelets and C4 Levels" within the table. 5) Appendix 1: Sirolimus Cohort Schedule of Activities: Remote Visit 3.1 Phone Contact, a phone contact was added for possible sirolimus dose level adjustment on Day 6 (+3 days) along with Footnote b "Sirolimus dose adjustments as soon as the trough concentration level from Day 4 is available." This information was included on the sirolimus dosing table within Appendix 1 but was inadvertently originally omitted from the sirolimus cohort Schedule of Activities. 6) Appendix 1: Sirolimus Cohort Schedule of Activities: "Sirolimus trough level" replaced with "Sirolimus trough level (locally)" This information was included on the sirolimus dosing table within Appendix 1 but was inadvertently originally omitted from the sirolimus cohort Schedule of Activities. 7) Appendix 1: Sirolimus Cohort Schedule of Activities: For remote visits 4.2 and 5.2, the following sentence was added as footnote C: "Unless clinical concern and/or subject

		preference warrants an in-person visit, remote visits may be performed." This sentence was added to allow for flexibility so an in person visit is allowable. 8) Appendix 1: Sirolimus Cohort: Cohort Intervention: Added guidance on how to calculate the BSA for the sirolimus dose when required based on the subject's weight. 9) Added Appendix 3: Protocol Amendment History 10) Appendix 4 Abbreviations: Added additional abbreviations.

Appendix 4. Abbreviations

This following is a list of abbreviations that may be used in the protocol.

Abbreviation	Term
%pFVC	percent predicted forced vital capacity
4SC	Four Stair Climb
6MWD	six minute walk distance
6MWT	Six Minute Walk Test
AAV	adeno-associated virus
AAV9	adeno-associated virus, serotype 9
ACE	angiotensin converting enzyme
ADA	anti-drug antibodies
ADLs	activities of daily living
AE	adverse event
aHUS	atypical hemolytic uremic syndrome
ALT	alanine aminotransferase
ALP	alkaline phosphatase
ANA	Antinuclear antibody
ANC	absolute neutrophil count
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
ATS	American Thoracic Society
BBS	Biospecimen Banking System
BMD	Becker muscular dystrophy
BP	blood pressure
BSA	Body surface area
C4	Complement 4
C5b-9	Complement 5b-9
CDC	Center for Disease Control
cDNA	complementary DNA
CE	clinical evaluator
CINRG	Cooperative International Neuromuscular Research Group
CIOMS	Council for International Organizations of Medical Sciences
CK	creatine kinase
CK-MB	creatine kinase myocardial b fraction
CMV	Cytomegalovirus
CNS	central nervous system
COVID-19	Coronavirus disease 19
CR	cysteine-rich
CRF	case report form
CRP	C-reactive protein
CSA	clinical study agreement
C-SSRS	Columbia Suicide Severity Rating Scale
CT	c-terminal
cTnI	cardiac troponin I

Abbreviation	Term
CYP3A4	Cytochrome P450 3A4
DAP	Dystrophin-associated protein
DILI	drug-induced liver injury
DGC	Dystrophin-associated glycoprotein complex
DMD	Duchenne muscular dystrophy
DNA	deoxyribonucleic acid
EC	ethics committee
ECG	Electrocardiogram
E-DMC	external data monitoring committee
EDP	exposure during pregnancy
EDTA	edetic acid (ethylenediaminetetraacetic acid)
ELISpot	enzyme-linked immunospot
ELISA	enzyme-linked immunosorbent assay
CCI	
EQ VAS	EuorQol visual analogue scale
ERS	European Respiratory Study
ESR	erythrocyte sedimentation rate
ET	Early Termination
EU	European Union
EudraCT	European Clinical Trials Database
FDA	Food and Drug Administration
FEV	forced expiratory volume
FIH	first-in-human
FIP	first-in-patient
FVC	forced vital capacity
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
GLDH	glutamate dehydrogenase
GMP	good manufacturing practice
HAV	hepatitis A virus
HBsAG	hepatitis B surface antigen
hCK	hybrid creatine kinase
HCVAb	hepatitis C antibody
HFI	hereditary fructose intolerance
HR	heart rate
HT	height
IA	interim analysis
IB	Investigator's Brochure
ICD	informed consent document
ICH	International Conference on Harmonisation
ID	Identification
IFA	Immunofluorescence assay

Abbreviation	Term
IHC	Immunohistochemistry
IM	Intramuscular
INFα	interferon alpha
INFβ	interferon beta
IgG	immunoglobulin G
IgM	immunoglobulin M
IND	investigational new drug application
INR	international normalized ratio
IP-10	Interferon gamma-induced protein 10
IRB	institutional review board
IRT	interactive response technology
ITAC	Interferon-inducible T-cell alpha chemoattractant
ITR	inverted terminal repeats
IV	Intravenous
K_2 EDTA	dipotassium ethylenediaminetetraacetic acid
Kda	kilo-dalton
Kg	Kilogram
LC-MS	liquid chromatography/mass spectrometry
LFT	liver function test
LSLV	last subject last visit
LVEF	left ventricular ejection fraction
MenACWY	meningococcal conjugate
MenB	serogroup B meningococcal
MHP	mental health provider
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MSW	Master's level Clinical Social Workers
N	Number
N/A	not applicable
NAb	neutralizing antibodies
NOAEL	no observed adverse effect level
NSAA	North Star Ambulatory Assessment
qPCR	quantitative polymerase chain reaction
QTcF	Fridericia's correction formula
PACL	protocol administrative change letter
PCD	primary completion date
PD	pharmacodynamics
PEFR	peak expiratory flow rate
PFT	pulmonary function test
P-gp	P-glycoprotein
PI	principal investigator
PICC	peripherally inserted central catheter
PK	pharmacokinetic(s)
PNP	psychiatric nurse practitioners

Abbreviation	Term
CCI	
P-selectin: protein-selectin	P(platelet)-selectin glycoprotein ligand-1
PT	prothrombin time
PUL	Performance of Upper Limb
rAAV	Recombinant AAV
RBC	red blood cell
RNA	ribonucleic acid
ROA	route of administration
SAE	serious adverse event
SAP	statistical analysis plan
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SD	Standard deviation
SMA	Spinal muscular atrophy
SoA	schedule of activities
SOC	system organ class
SOP	standard operating procedure
SRSD	single reference safety document
SSID	single subject's identification
TBili	total bilirubin
TEAEs	treatment emergent AEs
temp	temperature
TG	Transgene
ULN	upper limit of normal
UL-PROM	Upper Limb Patient Reported Outcome Measure
US	United States
USPI	United States Package Insert
Vg	vector genomes
WB	Western blot
Wt	weight
CCI	

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