

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**212154Orig1s000**

**CLINICAL REVIEW(S)**

Clinical Review

Veneeta Tandon, Xiang Ling, Baikuntha Aryal, Ashutosh Rao  
Viltolarsen, NDA 212154

**CLINICAL REVIEW**

<b>Application Type</b>	NME
<b>Application Number(s)</b>	212154
<b>Priority or Standard</b>	Priority
<b>Submit Date(s)</b>	12/12/19
<b>Received Date(s)</b>	12/12/19
<b>PDUFA Goal Date</b>	8/12/20
<b>Division/Office</b>	DN1
<b>Reviewer Name(s)</b>	Veneeta Tandon (Clinical), Xiang Ling (Biometrics), Baikuntha Aryal, Ashutosh Rao (Dystrophin Bioassays)
<b>Review Completion Date</b>	
<b>Established/Proper Name</b>	Viltolarsen
<b>(Proposed) Trade Name</b>	VILTEPSO
<b>Applicant</b>	Nippon Shinyaku Co., Ltd.
<b>Dosage Form(s)</b>	Single use vial for IV infusion
<b>Applicant Proposed Dosing Regimen(s)</b>	80 mg/kg once a week
<b>Applicant Proposed Indication(s)/Population(s)</b>	Treatment of patients with Duchenne muscular Dystrophy (DMD) who have a confirmed mutation of the DMD gene that is amenable to exon 53 skipping
<b>Recommendation on Regulatory Action</b>	Accelerated Approval
<b>Recommended Indication(s)/Population(s) (if applicable)</b>	Treatment of patients with Duchenne muscular Dystrophy (DMD) who have a confirmed mutation of the DMD gene that is amenable to exon 53 skipping

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## Glossary

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AE	adverse event
BRF	Benefit Risk Framework
CDER	Center for Drug Evaluation and Research
CFR	Code of Federal Regulations
CRF	case report form
CRT	clinical review template
CSR	clinical study report
CSS	Controlled Substance Staff
DMC	data monitoring committee
ECG	electrocardiogram
FDA	Food and Drug Administration
FDASIA	Food and Drug Administration Safety and Innovation Act
GCP	good clinical practice
GRMP	good review management practice
ICH	International Council for Harmonization
IND	Investigational New Drug Application
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent to treat
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat
NDA	new drug application
NME	new molecular entity
OPQ	Office of Pharmaceutical Quality
OSI	Office of Scientific Investigation
PI	prescribing information or package insert
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PP	per protocol
PRO	patient reported outcome
SAE	serious adverse event
SAP	statistical analysis plan
SOC	standard of care
TEAE	treatment emergent adverse event
WRO	Written Response Only

## 1. Executive Summary

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### 1.1. Product Introduction

Drug and Indication: VILTEPSO® (also known as Viltolarsen, NS-065/NCNP-01) is a new molecular entity that is proposed for the treatment of Duchenne muscular dystrophy (DMD) in patients who have a confirmed mutation of the DMD gene that is amenable to exon 53 skipping of the dystrophin pre-messenger ribonucleic acid (pre-mRNA).

Viltolarsen binds to a specific sequence in exon 53 of the human dystrophin pre-mRNA that alters the exon/intron splicing patterns by skipping over exon 53 during splicing of pre-mRNA. This converts the DMD patient's out-of-frame mRNA into an in-frame Becker-like mRNA. Restoration of the open reading frame allows the generation of an internally truncated dystrophin that is partially functional. Viltolarsen is thought to be effective on DMD patients with exon deletions amenable to skipping of exon 53, such as 43-52, 45-52, 47-52, 48-52, 49-52, 50-52, or 52, which combined, is 8-10 % of all DMD patients.

Pharmacological Class: Viltolarsen is an antisense phosphorodiamidate morpholino oligonucleotide.

Dosage Form: VILTEPSO® is proposed to be available as a sterile drug formulation for intravenous infusion seen as a colorless clear solution filled in a clear glass vial. Each vial will contain 250 mg viltolarsen in 5 mL saline solution (50 mg/mL).

Proposed Regimen: 80 mg/kg once a week

### 1.2. Conclusions on the Substantial Evidence of Effectiveness

The Applicant proposes dystrophin as a surrogate endpoint that is reasonably likely to predict a clinical benefit for the approval of viltolarsen under the Accelerated Approval pathway. The review concludes that a statistically significant increase in truncated dystrophin expression was observed in DMD patients with a genetic mutation amenable to exon 53 skipping after 24 weekly intravenous administration of both 40 and 80 mg/kg viltolarsen in 8 patients each, shown using adequately validated analytical methods of western blot (primary endpoint) and mass spectrometry (secondary endpoint). After 24 weeks of treatment with viltolarsen, the median increase in truncated dystrophin with 40 mg/kg/week and 80 mg/kg/week viltolarsen is 4.6% and 3.8% of normal, respectively, when analyzed using western blot methodology. Two DMD drugs (eteplirsen and golodirsen) have been approved by the Agency under the Accelerated Approval regulation (21 CFR Subpart H) for which a determination has been made that dystrophin as a

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surrogate marker is reasonably likely to confer clinical benefit with median increases in truncated dystrophin of 0.1 and 0.88% of normal after 48 weeks of treatment, respectively.

Although the applicant has not provided adequate empirical evidence to address that the levels of truncated dystrophin produced by viltolarsen is reasonably likely to confer clinical benefit, a determination that truncated dystrophin as produced by eteplirsen and golodirsen was reasonably likely to predict clinical benefit has been made at the Agency level; hence, I rely on this determination to support accelerated approval of viltolarsen. Viltolarsen also meets the other two criteria for accelerated approval: DMD being a serious life-threatening condition and viltolarsen confers meaningful advantage over available therapy. Therefore, I recommend that accelerated approval of viltolarsen (VILTEPSO®) NDA 212154 be granted based on the precedent of accelerated approval of eteplirsen and golodirsen. Please see Section 7.4 for the further discussion on the empirical evidence provided by the applicant in the application to support that dystrophin levels as seen by viltolarsen is reasonably likely to predict clinical benefit in DMD patients amenable to exon 53 skipping.

The applicant is seeking approval of only the 80 mg/kg/week dose of viltolarsen. I support the approval of this dose since a marginally higher amount of truncated dystrophin production was observed at the 80 mg/kg/week dose by mass spectrometry analysis method, a secondary endpoint. The median increase in truncated dystrophin by mass spectrometry was 1.7% and 1.9% of normal for the viltolarsen doses of 40 mg/kg/week and 80 mg/kg/week, respectively. No clinically significant difference was observed in the safety of these two doses, although the safety database was small.

### 1.3. **Benefit-Risk Assessment**

### **Benefit-Risk Integrated Assessment**

Viltolarsen is proposed for the treatment of Duchenne muscular dystrophy (DMD) in patients who have a confirmed mutation of the DMD gene that is amenable to exon 53 skipping of the dystrophin pre-messenger ribonucleic acid (pre-mRNA). Viltolarsen is an antisense phosphorodiamidate morpholino oligonucleotide designed to bind to a specific sequence in exon 53 of the dystrophin pre-mRNA transcript and block the exon/intron splicing of exon 53 ("exon-skipping"), leading to mature mRNA transcripts that lack exon 53 and thereby producing a truncated dystrophin.

DMD is a rare progressive X-linked neuromuscular disorder that occurs due to the absence of dystrophin protein in male pediatric patients. DMD is present at birth, but the disorder becomes apparent between ages 3-5 years. The loss of muscle strength in DMD is progressive, leading to loss of ambulation in the teens. Progressive loss of muscle strength leads to decline in respiratory function, cardiac complications and ultimately death typically in the third decade.

Currently VYONDYS 53<sup>®</sup> (Golodersen), a similar antisense oligonucleotide was granted accelerated approval in December 2019 based on increase in dystrophin expression for the same indication; however, the clinical benefit of this increase in dystrophin expression has not been established. In addition, EXONDYS 51<sup>®</sup> (Eteplirsen) was granted accelerated approval in September 2016 for mutations amenable to exon 51 skipping and EMFLAZA (deflazacort), a glucocorticoid with anti-inflammatory and immunosuppressive properties was approved for the treatment of DMD in patients 2 years and older.

This submission contains a dose-finding study 201 to assess increase in dystrophin and safety/tolerability in 16 DMD patients 4 to <10 years of age that were on stable doses of corticosteroids for ≥3 months. The initial 4 weeks of the study were randomized double-blind placebo controlled after which all patients received viltolarsen either 40 (N=8) or 80 mg/kg/week (N=8) for additional 20 weeks. The muscle biopsies for dystrophin assessment was collected from biceps muscle at baseline and Week 25. An ongoing open-label extension Study 202 provided long-term safety up to 107 weeks in these 16 patients. In addition, a similar 24-week study P1/2 in 16 patients provided safety data, however was not considered supportive of dystrophin expression due to inadequacy to dystrophin assessment methodology.

The applicant proposes dystrophin as a surrogate endpoint that is reasonably likely to predict a clinical benefit for approval under accelerated approval pathway and is seeking approval of the 80 mg/kg/week dose. The truncated dystrophin production with 20-24 weeks treatment with viltolarsen is shown in the following Table for both doses. Although the truncated dystrophin levels are similar between the two doses, the 80 mg/kg/week may be appropriate for approval based on marginally higher amounts of dystrophin by mass spectrometry method.

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Truncated Dystrophin (% of Normal)	Parameter	40 mg/kg/week	80 mg/kg/week
Western Blot	Mean ± SD baseline	0.3 ± 0.1	0.6 ± 0.82
	Mean ±SD at Week 25	5.7 ± 2.37	5.9 ± 4.50
	<b>Mean ± SD Change from baseline (p-value)</b>	<b>5.4 ± 2.4 (p=0.0004)</b>	<b>5.3 ± 4.5 (p=0.01)</b>
	<b>Median Change from baseline</b>	<b>4.6</b>	<b>3.8</b>
Mass spectrometry	Mean ± SD baseline	0.5 ± 0.15	0.6 ± 0.19
	Mean ±SD at Week 25	2.1 ± 1.09	4.2 ± 3.73
	<b>Mean ± SD Change from baseline (nominal p-value)</b>	<b>1.5 ± 1.1 (p=0.006)</b>	<b>3.7 ± 3.8 (p=0.03)</b>
	<b>Median Change from baseline</b>	<b>1.7</b>	<b>1.9</b>

Viltolarsen meets the accelerated approval criteria in terms of having the potential to address an unmet need in a serious and life-threatening disease and having an advantage over available therapies (1) Deflazacort, as not all DMD patients are on steroids and (2) VYONDYS 53®, that received accelerated approval where clinical benefit has not been established. However, there is uncertainty that the levels of dystrophin produced by viltolarsen would be reasonably likely to predict clinical benefit in patients as the Applicant has not provided conclusive evidence to suggest that median truncated dystrophin levels of 1.9-3.8% of normal (mass spectrometry and western blot methods) are reasonably likely to confer clinical benefit to patients. Despite this deficiency, accelerated approval of viltolarsen based on dystrophin as a surrogate is appropriate for viltolarsen based on two precedents in CDER where accelerated approval was granted with the conclusion that lower amounts of truncated dystrophin are reasonably likely to predict clinical benefit. A confirmatory study to confirm clinical benefit at the 80 mg/kg/week dose is ongoing.

Given, the rarity of the condition, the safety database of 32 patients was adequate to support the safety of viltolarsen in DMD. The most frequently observed adverse events included upper respiratory tract infection, cough, pyrexia, nasal congestion and injection site reactions. Kidney is a known target organ for antisense oligonucleotides. Nonclinical data suggest the potential for serious kidney toxicity in humans. However, no serious renal adverse reaction was reported in viltolarsen clinical studies. The seriousness of the indication along with the unmet medical need make the potential risk for kidney toxicity acceptable, and manageable through labeling with for monitoring for kidney toxicity.

**Benefit-Risk Dimensions**

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Dimension	Evidence and Uncertainties	Conclusions and Reasons
<a href="#">Analysis of Condition</a>	<ul style="list-style-type: none"> <li>DMD is a rare progressive X-linked neuromuscular disorder caused by mutations in the dystrophin gene that result in loss of muscle fibers, inflammation, and progressive replacement of muscle by fibrotic and adipose tissue.</li> <li>The progression of muscle weakness is proximal-to-distal which typically begins at age 3-5 years. By age 8-16, patients become wheel chair bound followed by progressive respiratory and cardiac abnormalities that lead to death before the age of 30 years.</li> <li>Exon 53 skip-amenable DMD, a subgroup of DMD is defined by the presence of dystrophin exon 53 and the deletion of one or more exons contiguous with exon 53, resulting in an out-of-frame deletion in which the reading frame is restorable by the skipping (removing) of exon-53.</li> <li>Mutations amenable to exon 53 skipping are thought to comprise 8%-10% of the DMD population.</li> </ul>	<p>The loss of muscle strength in DMD is progressive leading to loss of ambulation in the teens. Progressive loss of muscle strength leads to decline in respiratory function, cardiac complications and ultimately death typically in the third decade.</p>
<a href="#">Current Treatment Options</a>	<ul style="list-style-type: none"> <li>VYONDYS 53<sup>®</sup> (Golidersen) is an FDA approved treatment specific for DMD patients amenable to exon 53 skipping similar to the proposed indication for viltolarsen.</li> <li>EMFLAZA<sup>®</sup> (Deflazacort) is a glucocorticoid approved for treatment of DMD in patients 2 years of age and older.</li> <li>EXONDYS 51<sup>®</sup> (Eteplirsen) is approved for the treatment of DMD patients amenable to exon 51 skipping</li> </ul>	<p>There is substantial unmet need for therapies in DMD patients amenable to exon 53 skipping as the clinical benefit of approved treatment for the same indication (VYONDYS 53<sup>®</sup>) has not established a clinical benefit in these patients. In addition, there are many patients that do not use steroids due to its safety profile.</p>
<a href="#">Benefit</a>	<ul style="list-style-type: none"> <li>Percent of normal truncated dystrophin quantification by western blot of biceps brachii muscle biopsies showed a mean increase in dystrophin levels from 0.30% and 0.6% of normal at baseline to 5.7 and 5.9% of normal after 24 weeks of treatment with viltolarsen 40 and 80 mg/kg/week, respectively. The <u>mean change from baseline</u> in dystrophin level was 5.4% (p&lt;0.001) and 5.3% (P=0.01) of normal for the 40 and 80 mg/kg/week respectively with western blot analysis. The <u>median change in dystrophin</u> was 4.6% and 3.8% of normal for viltolarsen 40 and 80 mg/kg/week, respectively.</li> </ul>	<p>A statistically significant increase in truncated dystrophin was observed at both 40 and 80 mg/kg/week doses of viltolarsen by both Western blot (primary endpoint), and mass spectrometry (secondary endpoint), with a slight trend of higher dystrophin at 80 mg/kg/week with mass spectrometry. Based on the empirical evidence provided by the applicant it is uncertain that the levels of truncated dystrophin produced will confer clinical benefit to the patients; however, there is</p>

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Dimension	Evidence and Uncertainties	Conclusions and Reasons
	<ul style="list-style-type: none"> <li>Percent of normal truncated dystrophin quantification by mass spectrometry showed a mean increase in dystrophin levels from 0.5% and 0.6% of normal at baseline to 1.5 and 3.7% of normal after 24 weeks of treatment with viltolarsen 40 and 80 mg/kg/week, respectively. The <u>mean change from baseline</u> in dystrophin level was 1.5% (p=0.006) and 3.7% (P=0.03) of normal for the 40 and 80 mg/kg/week respectively with mass spectrometry analysis. The <u>median change in dystrophin</u> was 1.7% and 1.9% of normal for viltolarsen 40 and 80 mg/kg/week, respectively.</li> <li>Although amount of truncated dystrophin is similar if not higher at the 40 mg/kg/wk dose, it may be appropriate to approve 80 mg/kg/week dose based on slight trend of higher amounts with mass spectrometry analysis.</li> <li>Exon 53 skipping was confirmed by measurement and sequence verification of exon 53 skipped mRNA. There was an increase in exon 53 skipping in all patient samples.</li> </ul>	<p>precedent in CDER where truncated dystrophin in amounts lower (0.1 and 0.9% of normal) than that produced by viltolarsen was concluded to predict clinical benefit in DMD patients.</p>
<p><a href="#">Risk and Risk Management</a></p>	<ul style="list-style-type: none"> <li>A total of 32 DMD patients were exposed to viltolarsen 40 and 80 mg/kg/wk for 20-24 weeks. A total 16 of these 32 patients were exposed to viltolarsen for durations &gt;1 year in an ongoing study.</li> <li>Most common TEAEs (incidence ≥10%) were upper respiratory tract infection, cough, pyrexia, nasal congestion and injection site reactions. All injection site reactions were mild.</li> <li>Renal toxicity was the primary toxicity observed in nonclinical studies. No serious renal adverse reaction or clinically interpretable renal abnormalities were observed in the clinical studies. The safety database is small and cannot assure absence of risk of renal toxicity in patients, therefore potential of risk and monitoring strategies should be included in the product labeling.</li> <li>There is inadequate data to assess the potential for QT prolongation.</li> </ul>	<p>Most frequent adverse events were mild and included upper respiratory tract infection, cough, pyrexia, nasal congestion and injection site reactions.</p> <p>No serious renal adverse reaction was observed in the clinical studies, but nonclinical data indicate a potential for serious renal toxicity in humans. A Warning and Precaution should be included in the product labeling regarding potential renal toxicity with the enhanced monitoring for such toxicity.</p> <p>The applicant should be required to evaluate the potential for QT prolongation in the confirmatory study as post-marketing requirements.</p>

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### 1.4. Patient Experience Data

Patient Experience Data Relevant to this Application (check all that apply)

<input type="checkbox"/>	The patient experience data that was submitted as part of the application include:	Section where discussed, if applicable
<input type="checkbox"/>	Clinical outcome assessment (COA) data, such as	[e.g., Sec 6.1 Study endpoints]
<input type="checkbox"/>	<input type="checkbox"/> Patient reported outcome (PRO)	
<input type="checkbox"/>	<input type="checkbox"/> Observer reported outcome (ObsRO)	
<input type="checkbox"/>	<input type="checkbox"/> Clinician reported outcome (ClinRO)	
<input type="checkbox"/>	<input type="checkbox"/> Performance outcome (PerfO)	
<input type="checkbox"/>	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)	
<input type="checkbox"/>	Patient-focused drug development or other stakeholder meeting summary reports	[e.g., Sec 2.1 Analysis of Condition]
<input type="checkbox"/>	Observational survey studies designed to capture patient experience data	
<input type="checkbox"/>	Natural history studies	
<input type="checkbox"/>	Patient preference studies (e.g., submitted studies or scientific publications)	
<input type="checkbox"/>	Other: (Please specify)	
<input type="checkbox"/>	Patient experience data that were not submitted in the application, but were considered in this review:	
<input type="checkbox"/>	<input type="checkbox"/> Input informed from participation in meetings with patient stakeholders	
<input type="checkbox"/>	<input type="checkbox"/> Patient-focused drug development or other stakeholder meeting summary reports	[e.g., Current Treatment Options]
<input type="checkbox"/>	<input type="checkbox"/> Observational survey studies designed to capture patient experience data	
<input type="checkbox"/>	<input type="checkbox"/> Other: (Please specify)	
<input checked="" type="checkbox"/>	Patient experience data was not submitted as part of this application.	

## 2. Therapeutic Context

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### 2.1. Analysis of Condition

Duchenne muscular dystrophy (DMD) is a X-linked recessive disorder caused by mutations in the dystrophin gene. It is the most frequent of the early onset muscular dystrophies that occur almost exclusively in males. A small percentage of female carriers may exhibit a range of muscle symptoms from the full Duchenne phenotype to milder skeletal muscle weakness. Exon 53 skip-amenable DMD, a subgroup of DMD is defined by the presence of dystrophin exon 53 and the deletion of one or more exons contiguous with exon 53, resulting in an out-of-frame deletion in which the reading frame is restorable by the skipping (removing) of exon-53.

**Etiology:** DMD is caused by the absence or near absence of functional dystrophin protein due to mutations in the DMD gene. In normal striated muscle, the cytoplasmic dystrophin protein links intracellular actin with the extracellular matrix to provide structural stability of the muscle cell membrane. Mutations that disrupt the translational reading frame of the dystrophin transcript, lead to a prematurely aborted dystrophin synthesis. Mutations due to out-of-frame amino acid translation caused most commonly by a deletion of 1 or more exons from the dystrophin gene result in premature truncation of dystrophin translation which produces nonfunctional and unstable dystrophin proteins.

Lack of dystrophin results, through mechanisms not precisely understood, in degeneration of muscle fibers, attracting inflammatory cells and ultimately replacement by fibrotic tissue and adipose tissue. Dystrophin deficiency results in loss of neuronal nitric oxide synthase, which normally is localized to the sarcolemma as part of the dystrophin–glycoprotein complex. The absence of functional dystrophin in DMD results in deterioration of the skeletal musculature with subsequent loss of strength and function.

**Clinical Features:** DMD is present at birth, but the disorder usually becomes apparent between ages 3 and 5 years. There is a proximal-to-distal progression of muscle weakness. The boys fall frequently. Running, jumping, and hopping are invariably abnormal. By age 5 years, muscle weakness is obvious by muscle testing. On getting up from the floor, the patient uses his hands to climb up himself. Contractures of the heel cords and iliotibial bands become apparent by age 6 years, when toe walking is associated with a lordotic posture. Loss of muscle strength is progressive, with predilection for proximal limb muscles and the neck flexors; leg involvement is more severe than arm involvement. Between ages 8 and 10 years, walking may require the use of braces. By age 8-16, patients become wheel chair bound. Contractures become fixed, and a progressive scoliosis often develops. The chest deformity with scoliosis impairs pulmonary function, which is already diminished by muscle weakness. By age 16–18 years, patients are predisposed to serious, sometimes fatal pulmonary infections. In the last years of life the patient becomes bedfast. In general, there is a wide range of functional ability at a given age. The use of

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glucocorticoids and the management of spine deformity, pulmonary and cardiac dysfunctions have altered the timing of some of the clinical milestones of the disease.

**Life Span:** Patients with DMD usually survive until late adolescence but not more than 20 to 25 percent live beyond the twenty-fifth year. Respiratory, orthopedic and cardiac complications emerge, and without intervention the mean age at death is around 19 years. Following the introduction in the 1990s of assisted ventilation in the later stages of the disease, the mean age of survival (for those ventilated patients who do not develop early and severe cardiomyopathy) shifted to 24 years, with some surviving to the early thirties.

**Incidence:** The incidence of DMD is about 1 in 5000 live male births globally. Prevalence of DMD has been reported as approximately 16 cases per 100,000 live male births in the United States (US).<sup>1</sup> Exon 53 skipping would be applicable to approximately 8% to 10% of DMD patients.<sup>2,3</sup>

**Diagnostic Criteria:** All boys with a clinical suspicion of a DMD diagnosis are subjected to molecular analysis of their dystrophin gene. Molecular methods that assess DNA copy number are used as the initial step in the diagnosis of DMD. If no deletions are identified, then DNA sequencing is performed to identify point mutations or small insertions or deletions. Three commonly used tests to determine a patient's mutation in the dystrophin gene include Multiplex Ligation-dependent Probe Amplification (MLPA), High-density Array Comparative Genomic Hybridization, and Single-Condition Amplification Internal Primer Sequencing. Serum CK levels are invariably elevated to between 20 and 100 times normal. The levels are abnormal at birth but decline late in the disease because of inactivity and loss of muscle mass. EMG demonstrates features typical of myopathy.

## 2.2. Analysis of Current Treatment Options

The approved therapies are summarized in Table 1.

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<sup>1</sup> Ryder S, et al. The burden, epidemiology, costs and treatment for Duchenne muscular dystrophy: an evidence review. *Orphanet J Rare Dis* 2017;12:79.

<sup>2</sup> Aartsma-Rus A et al. Theoretic applicability of antisense-mediated exon skipping for Duchenne muscular dystrophy mutations. *Hum Mutat.* 2009 Mar 1;30(3):293-9.

<sup>3</sup> Bladen CL, et al. The TREATNMD DMD Global Database: analysis of more than 7,000 Duchenne muscular dystrophy mutations. *Hum Mutat.* 2015;36(4): 395-402.

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**Table 1 Summary of Approved Treatments.**

Product (s) Name	Relevant Indication	Year of Approval	Route and Frequency of Administration	Efficacy Information	Important Safety and Tolerability Issues	Other Comments (e.g., subpopulation not addressed)
FDA Approved Treatments for mutations amenable to Exon 53 skipping						
None						
Other Treatments						
Deflazacort (EMFLAZA) N208684, 208685	DMD	2017	Oral 0.9 mg/kg/day	N=196 Placebo-controlled Randomized, double-blind 12-week study with 2 doses), re-randomized to active comparator for additional 40 weeks, primary endpoint was muscle strength graded by Medical Research Council (MRC) 11-point scale	Stunted growth, weight gain	Approved for $\geq 2$ years
Eteplirsen (EXONDYS 51) N 206488	DMD mutation amenable to exon 51 skipping	2016 Accelerat-ed approval	IV infusion 30 mg/kg once weekly	N=12 The median increase in dystrophin of 0.10% after 48 weeks	Balance disorder and vomiting	
Golidersen (VYONDYS) N211970	DMD mutation amenable to exon 53 skipping	2020 (Accelara-ted approval	IV infusion 30 mg/kg once weekly	N=25 The median increase in dystrophin of 0.88% after 48 weeks	Monitoring for Renal toxicity and drug hypersensitivity	

## 3. Regulatory Background

### 3.1. U.S. Regulatory Actions and Marketing History

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Viltolarsen is a new molecular entity and is not currently marketed in the US.

### 3.2. Summary of Presubmission/Submission Regulatory Activity

A brief chronology of the regulatory activity with the applicant regarding efficacy and safety related discussions during the development of viltolarsen and additional important milestones is tabulated below. The regulatory interactions regarding other review disciplines will be addressed in the respective reviews (i.e., chemistry, and nonclinical).

Date	Summary of Regulatory Activity
20 October 2015	<p>Pre-IND meeting</p> <ul style="list-style-type: none"><li>• Agency recommended adequate dose-finding study to establish the Maximum tolerated dose (MTD)</li><li>• Agreed on starting dose of 40 mg/kg/week, but unclear if 80 mg/kg/week would be the MTD</li><li>• Recommended conducting placebo-controlled study with 2 doses and adequate statistical power after MTD study is performed.</li><li>• Agreed dystrophin expression as primary endpoint in a dose-finding Phase 2A study.</li><li>• Agreed that the proposed age range of 4 to &lt;8 years is acceptable but found that the applicant's argument that biomarkers results would be most clear in this specific age to be theoretical and encouraged a wider age range to be studied.</li><li>• Agency advised that historically controlled studies would unlikely be able to provide substantial evidence of efficacy.</li><li>• Agency agreed that Time to Stand (TTSTAND) would be an acceptable endpoint in a Phase 2B placebo-controlled study to support full approval or in a confirmatory study if accelerated approval is based on dystrophin expression.</li></ul>
25 October 2016	Fast Tract Designation granted
12 January 2017	Orphan Drug Designation granted
24 January 2017	Rare Pediatric Disease Designation granted
3 July 2017	<p>Type C WRO</p> <ul style="list-style-type: none"><li>• The applicant was advised that in the rare disease population where patient resources are critical, the applicant should consider the conduct of a well-designed, randomized, placebo controlled double blind study of at least 48 weeks duration.</li><li>• Dystrophin expression can be the primary endpoint, but the study should be of sufficient size and duration to be able to evaluate clinical efficacy and establish a correlation between</li></ul>

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	any changes in dystrophin expression and trial's clinical endpoint
	(b) (4)
15 May 2018	Type C meeting: <ul style="list-style-type: none"><li>• Applicant requested the Division's feedback regarding the appropriateness of an NDA submission for viltolarsen to be considered under the accelerated approval pathway and the confirmatory trial design</li><li>• Agency agreed that the US Phase 2 study and supporting data from Japan Phase1/2 Study, if based on scientifically sound design and rigorous analytical method could serve the basis of accelerated approval.</li><li>• Agency advised that a future NDA submission must present evidence that the dystrophin data are reasonably likely to predict clinical benefit.</li><li>• The applicant was reminded that the confirmatory trial should be underway at the time of NDA submission.</li></ul>
September 2018	Pre-NDA meeting: <ul style="list-style-type: none"><li>• Discussed the content and format of NDA.</li><li>• Agency reiterated that a confirmatory placebo-controlled study should be ongoing at the time of NDA submission</li></ul>
15 January 2019	Rolling Review granted with non-clinical as Part 1 of the submission
30 September 2019	Rare pediatric disease priority review voucher received

### 3.3. Foreign Regulatory Actions and Marketing History

Viltolarsen is approved in Japan.

## 4. Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

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### 4.1. Office of Scientific Investigations (OSI)

None

### 4.2. Product Quality

None

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### 4.3. **Nonclinical Pharmacology/Toxicology**

Renal tubule injury was the primary toxicity in adult male mice, juvenile mice and monkeys. Kidney toxicity resulted in unscheduled death of two at the highest dose and was characterized by slight increases in urea nitrogen and creatinine and increases in kidney weight, histologic findings of intratubular dilation and epithelial vacuolation in the distal tubule and collecting duct in the middle and high dose groups in adult mice. Primary toxicity in juvenile male mice included renal tubule vacuolation, dilation, and degeneration; and in monkeys included epithelial vacuolation and dilation of the proximal tubules in high dose group.

Please refer to Nonclinical review for details.

### 4.4. **Clinical Pharmacology**

None

## **5. Sources of Clinical Data and Review Strategy**

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### **5.1. Table of Clinical Studies**

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**Listing of Clinical Trials Relevant to this NDA/BLA**

Trial Identity	Trial Design	Regimen/ schedule/ route	Study Endpoints	Treatment Duration/ Follow Up	No. of patients enrolled in each arm	Study Population
<b>Controlled Clinical Studies</b>						
<b>NS-065-201</b> (NCT02740972)  (US/Canada)  Primary Efficacy/Safety	DB, PC 4-week initial dosing period; 20-week OL period; natural history control for strength and function tests; DF	Viltolarsen injection 250 mg, IV, 40 or 80 mg/kg/wk, for 20 or 24 weeks; matching placebo for initial 4 weeks	Primary: Dystrophin  Natural history control for strength and function tests	4 weeks Controlled 20 weeks Uncontrolled	Viltolarsen 40 mg/kg/wk=6 80 mg/kg/wk=5 Placebo=5  20-weeks OL: Viltolarsen 40 mg/kg/wk=8 80 mg/kg/wk=8	DMD boys ≥4 to < 10 years  N=16
<b>Uncontrolled Clinical Studies</b>						
<b>NS-065-202</b> (NCT03167255)  (US/Canada)  <b>Long term Safety OL Extension of NS-065-201</b>	OL, natural history control for strength and function tests	Viltolarsen injection 250 mg, IV, 40 or 80 mg/kg/wk Up to 144 weeks (at least 73 weeks for initial NDA)	Long term Safety  Natural history control for strength and function tests	73 to 104 weeks	Viltolarsen 40 mg/kg/wk=8 80 mg/kg/wk=8 Ongoing	
<b>NS065/NCNP01-P1/2</b>  (Japan)  Supportive Efficacy and Safety	OL, uncontrolled	Viltolarsen injection 250 mg, IV, 40 or 80 mg/kg/wk	Dystrophin	24 weeks	Viltolarsen 40 mg/kg/wk=8 80 mg/kg/wk=8	DMD boys ≥5 to < 18 years  N=16
<b>NCNP/DMT01</b> (NCT02081625)	OL, uncontrolled	Viltolarsen injection 125 mg,	Dystrophin	12 weeks	Viltolarsen 1.25 mg/kg=3	DMD boys 5 to < 10

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(Japan) Proof-of-concept		IV; 1.25, 5, or 20 mg/kg/wk 12 weeks			5 mg/kg=3 20 mg/kg=4	years N=10
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## 5.2. Review Strategy

This is a combined review on the part of the Clinical, Biometrics, and Office of Biotechnology Products (Bioassay) Disciplines. Dr. Tandon reviewed the clinical safety and the efficacy results, Dr. Ling, the statistics associated with the primary endpoint, and Drs. Aryal and Rao, the methodology used for dystrophin mRNA and protein quantification (e.g., Western Blots, mass spectrometry Immunohistochemistry and RT-PCR techniques). Dr Tandon performed the risk-benefit analysis in this review. Consults were requested to the Division of Cardiology and Nephrology for advice on Renal toxicity associated viltolarsen and for the adequacy or QTc assessments to waive the applicant's request for a waiver for a Thorough QTc Study.

## 6. Review of Relevant Individual Trials Used to Support Efficacy

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### 6.1. Study NS-065/NCNP-01-201 (Referred as Study 201 in this review)

A Phase 2, Dose Finding Study to Assess the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of NS-065/NCNP-01 in ambulant boys ages 4 to <10 years with Duchenne Muscular Dystrophy (DMD) (clinicaltrials.gov identifier: NCT02740972)

#### 6.1.1. Study Design

##### Overview and Objective

##### Primary Objectives

- To evaluate the safety and tolerability of low (40 mg/kg/week) and high (80 mg/kg/week) intravenous (IV) doses of viltolarsen Injection in ambulant boys with DMD.
- To evaluate the effects of viltolarsen injection on induction of dystrophin protein in muscle after 20 to 24 weeks of treatment measured by Western blot.
- To evaluate the pharmacokinetics (PK)

##### Secondary Objectives

- To evaluate induction of dystrophin mRNA and protein in muscle after 20 to 24 weeks of treatment as measured by reverse-transcriptase-polymerase chain reaction (RT-PCR) for messenger ribonucleic acid (mRNA) analysis and immunofluorescence staining (IF) and mass spectrometry (MS) methods for protein analysis.
- To investigate the effect viltolarsen injection after 20 to 24 weeks of treatment on muscle strength, mobility, and functional exercise capacity, as measured by Time to Stand From Supine (TTSTAND), Time to Run/Walk 10 meters (TTRW), Time to Climb 4 Stairs

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(TTCLIMB), North Star Ambulatory Assessment (NSAA), 6-Minute Walk Test (6MWT) and Quantitative Muscle Testing (QMT) vs. a matched natural history control group.

### Exploratory Objective:

- To investigate the effects of low and high IV doses of viltolarsen injection on serum pharmacodynamic (PD) biomarkers.

### **Trial Design**

Population: A total of 16 ambulant boys ages 4 to <10 years with Duchenne Muscular Dystrophy (DMD)

This study was a multicenter, 2-period, dose-finding study of viltolarsen injection with sequentially enrolled dose cohorts:

- Low Dose 40 mg/kg/week
- High Dose 80 mg/kg/week.

Note: Doses will be addressed as 40 mg/kg/wk and 80 mg/kg/wk in the review.

Doses were administered once weekly by an IV infusion over a 1-hour period. Peripheral venous access (IV catheter that emptied into a peripheral vein in the arms, hands, legs, or feet). Central venous access (IV catheter that empties into a large central vein) was considered on a case-by-case basis.

The initial 4 weeks were a randomized, double-blind, placebo-controlled period to study acute safety of viltolarsen in 8 patients (3:1 randomization; 6 on viltolarsen and 2 on placebo). After a Screening Phase of Day -21 ( $\pm 7$ ), the 24-week Treatment Phase began on Day 1 with a Low Dose viltolarsen of 40 mg/kg/wk in Period 1. Study design schematic is shown in Figure 1.

All 8 patients were then dosed 40 mg/kg/wk for another 20 weeks starting Week 5 in Period 2. The patients that were on placebo had a total treatment duration of 20 weeks and those of viltolarsen had a total treatment duration of 24 week.

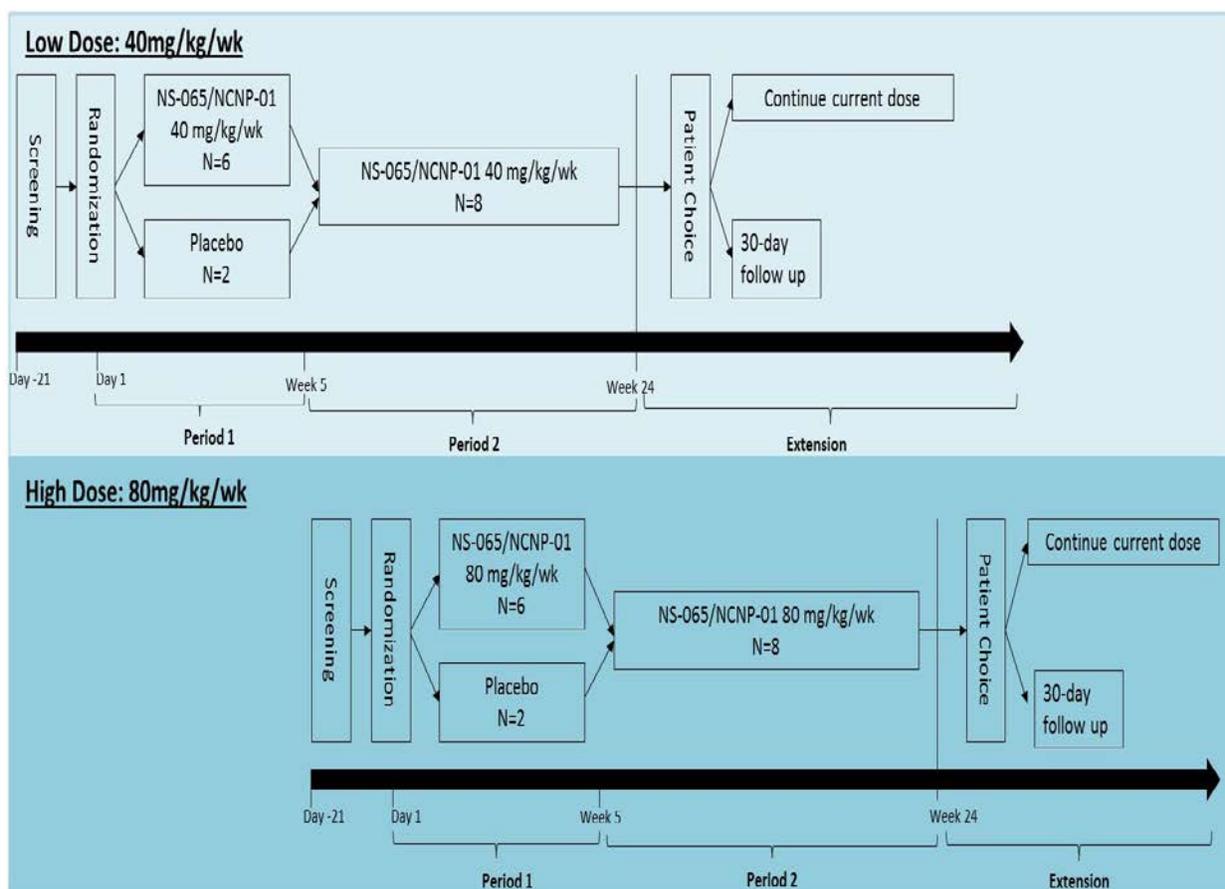
After 4 weeks of treatment with no safety signals for the entire Low Dose 40 mg/kg/wk cohort, the separate 80 mg/kg/wk cohort began with the same 3:1 viltolarsen:placebo ratio in 8 patients. However, the applicant notes that as the result of a re-ordering of blinded study drug at one site (randomization error), 5 patients received viltolarsen and 3 patients received placebo in Period 1 instead of 6 on viltolarsen and 2 on placebo.

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**Figure 1 Study Design Schematic**



Source: N212154 Clinical Study Report

Patients completing both the Low and High Dose 24-week treatment period were eligible for an open-label extension study under a separate protocol (NS-065/NCNP-01-202, referred as Study 202 in the review).

A Post-treatment Phase of 30-day interval (including Week 25) beginning after completion of the 24-week Treatment Phase and ending after a final phone call for collection of any information about adverse event (AE) and concomitant medications was planned for those patients who would not elect to enroll in the open-label extension study (Study 202).

*Primary Reviewer's Comment: This short placebo duration of as little as 2 weeks was agreed at the Pre-IND meeting (Oct 20, 2015)*

### Key Inclusion Criteria

The following were the criteria regarding the patient population:

- Patient had a confirmed diagnosis of DMD defined as:

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- a. Patient was male with clinical signs compatible with DMD; and
  - b. Patient had a confirmed DMD mutation(s) in the dystrophin gene that was amenable to skipping of exon 53 to restore the dystrophin mRNA reading-frame, including determination of unambiguous defined exon boundaries (using techniques such as Multiplex Ligation-dependent Probe Amplification, Comparative Genomic Hybridization array or other techniques with similar capability);
- Patient was  $\geq 4$  years at time of consent and  $<10$  years of age at time of first infusion in the study;
  - Patient was able to walk independently without assistive devices;
  - Patient was able to complete the TTSTAND, TTRW, and TTCLIMB assessments as at Screening
  - Patient was required to have been on a stable dose of glucocorticoid (GC) for at least 3 months prior to study entry, and was expected to remain on the stable dose of GC treatment for the duration of the study

### Exclusion Criteria

- Patient had experienced an acute illness within 4 weeks prior to the first dose of study medication, previous or ongoing medical condition, medical history, physical findings or laboratory abnormalities that could have affected safety
- Patient had evidence of symptomatic cardiomyopathy. (Note: asymptomatic cardiac abnormality on investigation was not exclusionary)
- Patient had severe behavioral or cognitive problems
- Patient had positive test results for hepatitis B antigen, hepatitis C antibody, or human immunodeficiency virus antibody at screening.
- Patient had positive test results for hepatitis B antigen, hepatitis C antibody, or human immunodeficiency virus antibody at screening
- Patient was taking any other investigational drug currently or within 3 months prior to the start of study treatment

### **Study Endpoints**

#### **Primary endpoint**

- Change from baseline in the measurement Dystrophin Protein by Western Blot Analysis at week 25

Muscle biopsies were taken from the biceps muscle of approximately 1 cm  $\times$  0.5 cm  $\times$  0.5 cm in size were surgically collected at the pre-treatment and post-treatment time points

#### **Secondary Endpoints**

- Induction of dystrophin protein in muscle measured by MS

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- Induction of dystrophin protein in muscle measured by IF-labeled antibody detection on tissue sections
- Induction of dystrophin mRNA in muscle measured by RT-PCR
- Time function Tests:
  - Time to Stand (TTSTAND) (measured in seconds),
  - Time to Climb 4 stairs (TTCLIMB) (measured in seconds),
  - Time to Run/Walk 10 meters test (TTRW) (measured in seconds),
- Six-minute Walk Test (6MWT) (measured in meters);
- North Star Ambulatory Assessment (NSAA 17-item test) combined scale
- Quantitative measures of strength were measured by CQMS (CINRG Quantitative Muscle System), and included: handgrip, isometric elbow flexion and extension, and knee flexion and extension (measured in pounds of pressure).
- Viltolarsen PK on Day 1, week 5 and Week 24

### Exploratory Endpoints:

- Serum pharmacodynamic biomarkers using SOMAScan assays.

### Statistical Analysis Plan (Dr. Xiang Ling)

The primary efficacy objective of the study was to evaluate the effects of low and high IV doses of viltolarsen injection on induction of dystrophin protein in muscle after 20-24 weeks of treatment. The primary efficacy endpoint was dystrophin protein in muscle measured by western blot. For each of the western blot tests, immunoblot dystrophin normalized to alpha-actin and immunoblot dystrophin normalized to myosin, 3 responses from 3 triplicate gels run were averaged to attain a single result for summarizing and analyzing. If any responses were missing, then the available non-missing responses were averaged.

Efficacy analyses were based on the modified Intent-to-Treat (mITT) population, consisting of all randomized patients who received at least 1 dose of investigational product and had a baseline assessment and at least 1 post baseline efficacy assessment. Patients were grouped by the two dose groups for efficacy analyses.

Western blot within-patient change in percentage of normal dystrophin production was tested using a paired t-test within each dose level. A two-sample t-test was used to compare change across the two dose levels. The two doses were also combined and tested using a paired difference t-test. Normality of change in percentage of normal dystrophin at post-baseline was to be assessed and if needed, a nonparametric test or a transformation to achieve normality would be performed.

There was no planned formal multiple testing procedure for secondary endpoints. Therefore, the secondary endpoints are considered exploratory and not included in statistical review.

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### Protocol Amendments

- **Amendment 2 – 14 October 2016 (Prior to First Patient’s First Visit)**
  - Changed the location of the muscle biopsy to the biceps from the anterior tibialis muscle.
- **Amendment 5 – 30 October 2017**
  - Changed primary objective of induction of dystrophin to be measured by Western blot.
  - Changed secondary objective of induction of dystrophin to be measured by MS.
- **Amendment 6 – 28 November 2017**
  - To maintain blinding during laboratory analysis of RT-PCR products, samples from all patients were tested using all 3 primer sets (exon 44+54/55, exon 46+54/55, and exon 48+54/55). Only the primer pair that provided the shortest RT-PCR product for each patient was used for the statistical analysis.

**Primary Reviewer’s Comment:** *These amendments will not bias the study results.*

### 6.1.2. Study Results

#### Compliance with Good Clinical Practices

The study was reported to have been conducted in accordance with the protocol, ICH and GCP regulatory requirements, the CFRs, FDA, and the current Declaration of Helsinki.

**Financial Disclosure** (See Appendix)

#### Patient Disposition

A total of 16 ambulatory patients participated in the study. There were no discontinuations in the study. Patient disposition is shown in Table 2. Group A are patients that were in the 4-week randomized portion of the study. Group B are patients in the 20-week open label portion of the study.

**Table 2 Patient Disposition**

	Treatment (mg/kg/wk)					Total N (%)
	Group A Placebo N (%)	Group A 40 mg/kg/wk N (%)	Group A 80 mg/kg/wk N (%)	Group B 40 mg/kg/wk N (%)	Group B 80 mg/kg/wk N (%)	
Number Screened						17
Number Randomized	5 (100)	6 (100)	5 (100)	8 (100)	8 (100)	16 (100)
Completed All Visits	5 (100)	6 (100)	5 (100)	8 (100)	8 (100)	16 (100)
Discontinued Study	0	0	0	0	0	0

Group A: In Period 1 of 4-week randomized part of the study

Group B: In Period 2 of 20-week open label extension part of the study

### Protocol Violations/Deviations

There were 6 major protocol violations were related to either 6MWT or CQMS not being completed on a few visits during the study. These protocol violations will have no impact on the evaluation of the primary endpoint.

### Table of Demographic Characteristics

Baseline demographic characteristics were similar across treatment groups as shown in Table 3

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**Table 3 Baseline Demographic Characteristics**

Baseline Demographic Characteristics	Treatments					
	Group A Placebo (N=5)	Group A 40 mg/kg/wk (N=6)	Group A 80 mg/kg/wk (N=5)	Group B 40 mg/kg/wk (N=8)	Group B 80 mg/kg/wk (N=8)	Total (N=16)
<b>Gender, n (%)</b>						
Male	5 (100)	6 (100)	5 (100)	8 (100)	8 (100)	16 (100)
Female	0	0	0	0	0	0
<b>Age (years)</b>						
Mean (SD)	7 (2)	7 (2)	7 (2)	7.5 (2)	7 (2)	7 (2)
Median	7	8	8	8	7	8
Minimum, maximum	4.9, 9.8	4.3, 9.8	4.8, 9.7	4.3, 9.8	4.8, 9.8	4.3, 9.8
<b>Race, n (%)</b>						
White	5 (100)	6 (100)	4 (80)	8 (100)	7 (88)	15 (94)
Black/African American	0	0	0	0	0	0
Asian	0	0	1 (20)	0	1 (12)	1 (6)
Other	0	0	0	0	0	0
<b>Ethnicity, n (%)</b>						
Hispanic or Latino	0	0	1 (20)	0	1 (12.5)	1 (6)
Not Hispanic or Latino	4 (80)	6 (100)	4 (80)	8 (100)	6 (75)	14 (88)
Not reported	1 (20)	0	0	0	1 (12.5)	1 (6)

Source: Primary reviewer's Analysis of ADSL.xpt

**Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)**

Key patient characteristics and Medical History at baseline were comparable as shown in Table 4

**Table 4 Other Baseline Disease Characteristics**

Baseline Demographic Characteristics	Treatments					
	Group A Placebo (N=5)	Group A 40 mg/kg/wk (N=6)	Group A 80 mg/kg/wk (N=5)	Group B 40 mg/kg/wk (N=8)	Group B 80 mg/kg/wk (N=8)	Total (N=16)
<b>Deleted Exons</b>						
45-52	3	2	2	2	5	7
47-52	0	1	0	1	0	1
48-52	1	0	2	1	2	3
49-52	1	1	1	2	1	3
50-52	0	2	0	2	0	2
<b>Age when the first signs or symptoms were identified (months)</b>						
Mean (SD)	42 (27)	34 (20)	31 (12)	34 (17)	38 (23)	36 (20)
Median	36	35	36	33	36	36
Minimum, maximum	12, 84	6, 60	18, 46	6,60	12,84	6, 84
<b>Age of independent walking (months)</b>						
Mean (SD)	15 (5)	17 (3)	23 (8)	17 (4)	19 (8)	18 (7)
Median	13	18	20	18	0	18
Minimum, maximum	12, 24	12, 19	16, 36	12,24	18	12, 36

Source: Primary reviewer's Analysis of ADMH.xpt

### Treatment Compliance, Concomitant Medications, and Rescue Medication Use

There were errors in the study drug not being administered appropriately on a single visit during the study, but all patients received all infusions. This is unlikely to impact the study results.

As required in the protocol all subjects were on daily administration of glucocorticoids. Majority of the patients were on deflazacort (4/5 in the placebo group, 5/6 in the 40 mg/kg/wk group and 3/5 in the 80 mg/kg/wk group). The remaining were on Prednisolone/prednisone. Other common medications were Vitamin D and Propofol used by all patients.

### Efficacy Results – Primary Endpoint

#### Dystrophin protein by Western Blot Analysis:

Western blot analysis on biceps muscle homogenate protein extract showed that viltolarsen resulted in increase in truncated dystrophin protein after 24 weeks weekly treatment of doses 40 and 80 mg/kg/wk.

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The primary analysis population for the evaluation of efficacy was the modified Intent-to-Treat (mITT) population that consisted of all randomized patients who received at least 1 dose of investigational product and have a baseline assessment and at least 1 post baseline efficacy assessment.

To control for differences in capillary loading and or muscle content in Western Blot analyses, a housekeeping protein that has presumed constant expression in muscle cells regardless of the disease state is used for normalization. To this effect, the proteins used in the Western blot analyses in this study included normalization to both myosin heavy chain and  $\alpha$ -actinin. Analytical methodology related to both are discussed in the subsequent section. Drs. Baikuntha and Rao conclude that analytical methodology for normalization with myosin heavy chain is relatively more reliable due to lesser variation in quantitation. However, I discuss results from both approaches. The baseline and post-treatment baseline dystrophin levels are summarized below for viltolarsen 40 and 80 mg/kg/wk groups and presented in Table 5:

**Table 5 Dystrophin by Western Blot (mITT Population)**

Visit/Statistics	Treatments							
	Normalized to Myosin (% of Normal)				Normalized to $\alpha$ -Actinin (% of Normal)			
	40 mg/kg/wk (N=8)		80 mg/kg/wk (N=8)		40 mg/kg/wk (N=8)		80 mg/kg/wk (N=8)	
	Obs	CFB	Obs	CFB	Obs	CFB	Obs	CFB
<b>Baseline</b>								
Mean (SD)	0.3 (0.1)	--	0.6 (0.8)	--	0.2 (0.2)	--	0.4 (0.7)	--
Median	0.3	--	0.4	--	0.1	--	0.2	--
Min, Max	0.1, 0.4	--	0.1, 2.6	--	0.0, 0.6	--	0.0, 2.1	--
<b>Week 25</b>								
Mean (SD)	5.7 (2.4)	5.4 (2.4)	5.9 (4.5)	5.3 (4.5)	5.4 (2.8)	5.2 (2.8)	3.7 (2.4)	3.3 (2.5)
Median	4.9	4.6	4.0	3.8	4.5	4.3	3.3	2.7
Min, Max	3.2, 10.3	2.8, 10.0	1.1, 14.4	0.7, 13.9	2.0, 10.4	1.7, 10.2	0.7, 8.0	0.3, 8.0
95% CI	--	(3.4, 7.4)	--	(1.6, 9.0)	--	(2.8,7.6)	--	(1.2, 5.3)
P-value (Paired T-Test)*	--	<b>0.0004</b>	--	<b>0.0123</b>	--	<b>0.0012</b>	--	<b>0.0074</b>
95% CI (80-40 mg)*	--	--	--	(-3.9, 3.7)	--	--	--	(-4.8, 0.9)
P-value (2-Sample Test)*	--	--	--	0.94	--	--	--	0.17

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CFB=change CI=confidence interval; Max=maximum; Min=minimum; mITT=modified intent-to-treat;  
Obs=observed; SD=standard deviation

For each visit where a biopsy was performed, up to 3 planned responses for each test were averaged to attain a single result for summarizing and analyzing.

\* Within-patient change from baseline was tested statistically using a paired t-test within each dose level to test change from baseline was different than 0. A two-sample t-test was used to test statistically whether the change from baseline in the 80 mg/kg/wk patients was different from the change from baseline in the 40 mg/kg/wk patients.

Source: Statistics Reviewer Analysis

### Baseline Dystrophin:

- **Normalized to myosin:** Mean baseline dystrophin protein measured by Western blot was 0.3% and 0.6% of normal in the viltolarsen 40 and 80 mg/kg/wk groups, respectively, when normalized to myosin (range 0.1- 2.6%).
- **Normalized to  $\alpha$ -actinin:** Mean baseline dystrophin protein measured by Western blot was 0.2% and 0.4% of normal in the viltolarsen 40 and 80 mg/kg/wk groups, respectively, when normalized to  $\alpha$ -actinin (range 0-2.1%).

### Post Treatment Dystrophin:

- **Normalized to myosin:** At Week 25, mean increases from baseline of 5.4% and 5.3% of normal compared with baseline in dystrophin were observed in the 40 and 80 mg/kg/wk groups: increases, respectively, when normalized to myosin (overall range 0.7-13.9%, irrespective of dose). The median increase from baseline was 4.6 and 3.8% of normal in the 40 and 80 mg/kg/wk groups, respectively, when normalized to myosin.
- **Normalized to  $\alpha$ -actinin:** At Week 25, mean increases from baseline of 5.2% and 3.3% of normal compared with baseline in dystrophin were observed in the 40 and 80 mg/kg/wk groups: increases, respectively, when normalized to  $\alpha$ -actinin (overall range 0.3- 10.2%, irrespective of dose). The median increase from baseline was 4.3 and 2.7% of normal in the 40 and 80 mg/kg/wk groups, respectively, when normalized to  $\alpha$ -actinin.

The mean change from baseline for the viltolarsen 40 and 80 mg/kg/wk groups were statistically significant whether normalized using myosin ( $p= 0.004$  and  $p=0.0123$ , respectively) or  $\alpha$ -actinin ( $p=0.0012$  and  $p=0.0074$ ) (See Table 5)

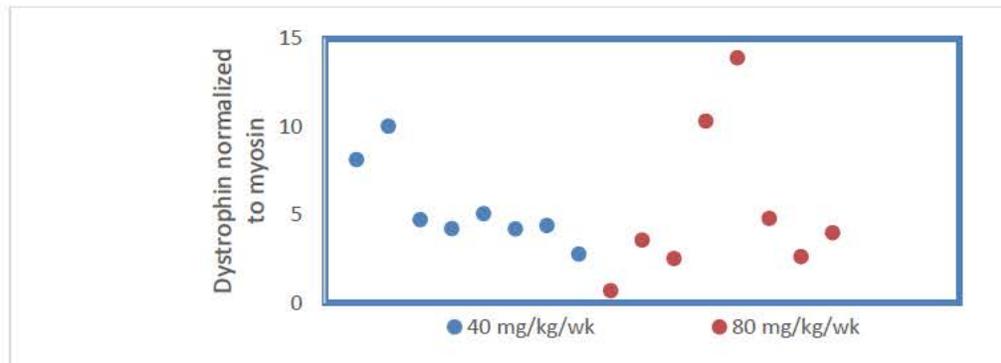
No statistically significant differences between dose groups were identified for dystrophin products by Western blot by either normalization by myosin or  $\alpha$ -actinin, as shown by a simple illustration with a scatter plot for the dystrophin normalized with myosin ( $p=0.94$ ).

Figure 2 shows the scatter plot for the individual percent of normal truncated dystrophin when normalized to myosin.

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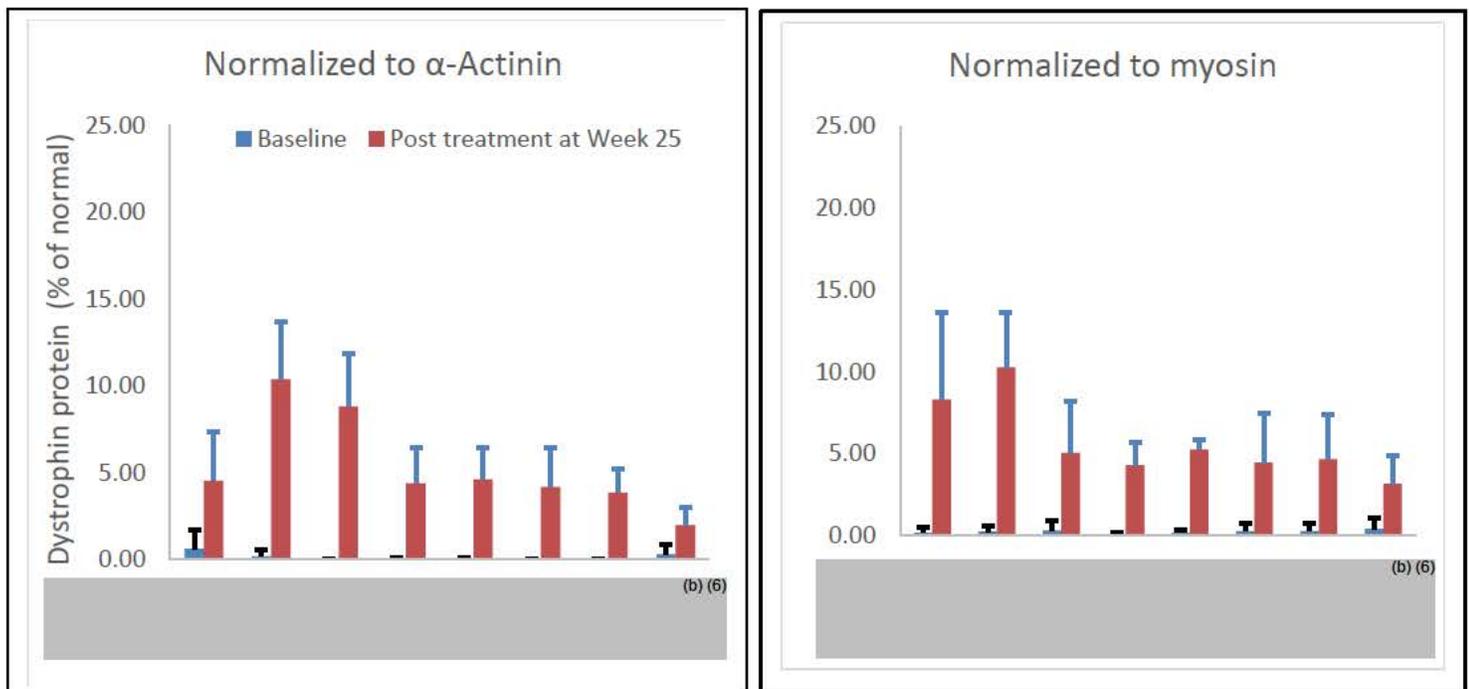
Figure 2 Percent of Normal Dystrophin normalized to myosin at 40 and 80 mg/kg/wk



Source: Primary Reviewer analysis

Western blot analyses were run in triplicate for the baseline and post-treatment biopsies. The individual patient Western blot analyses are presented in Table 6 for both  $\alpha$ -actinin and myosin normalized samples at both viltolarsen doses of 40 and 80 mg/kg/week. These data are graphically depicted in Figure 3 and Figure 4 for the 40 and 80 mg/kg/week doses, respectively.

Figure 3 Baseline and Post Treatment Dystrophin normalized for  $\alpha$ -actin and myosin for individual patients after treatment with 40 mg/kg/week viltolarsen

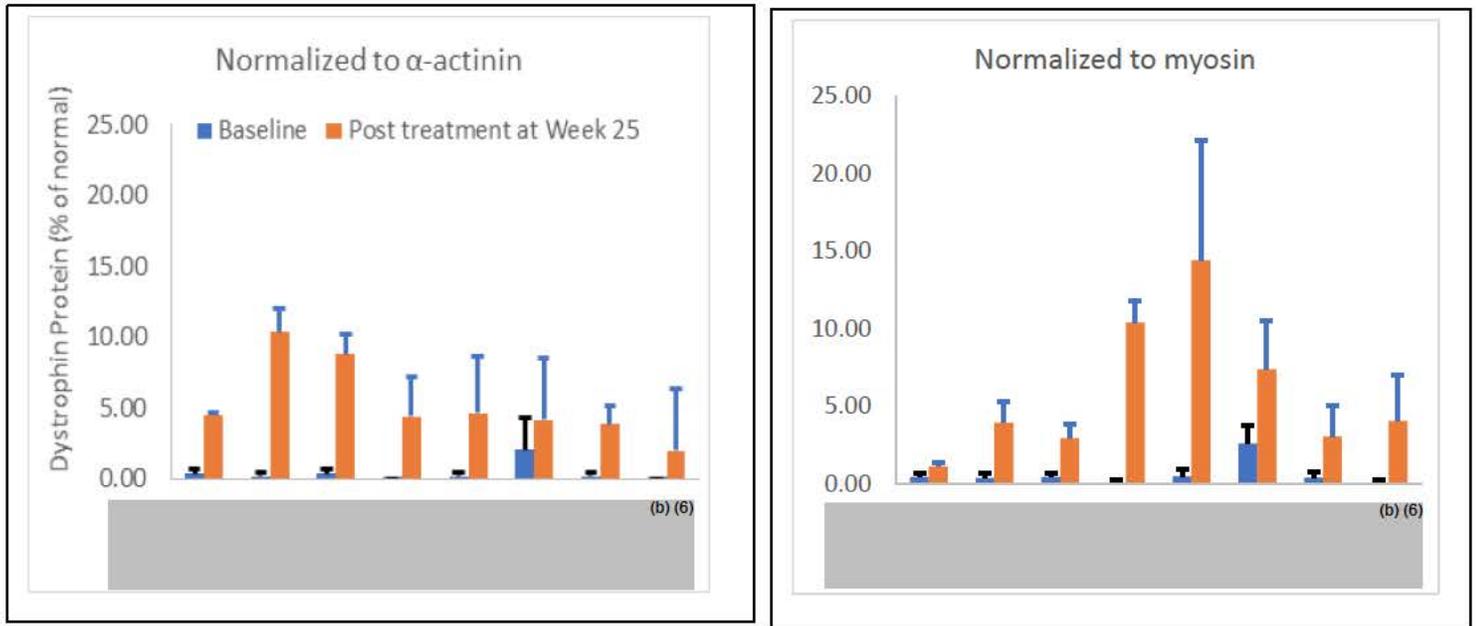


Source: Primary Reviewer analysis

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**Figure 4 Baseline and Post Treatment Dystrophin normalized for  $\alpha$ -actinin and myosin for individual patients after treatment with 80 mg/kg/week viltolarsen**



**Table 6 Triplicate Western Blot Analyses of individual Patient's**

**A: Normalization by  $\alpha$ -Actinin**

Subject	Dose	Baseline 1	Baseline 1	Baseline 1	Baseline Average	SD	Week 25 1	Week 25 2	Week 25 3	Week 25 Average	SD	Change from baseline
(b) (6)	40 mg	0	1.85	0	0.62	1.07	1.58	4.94	7.14	4.55	2.80	3.94
	40 mg	0	0.59	0	0.20	0.34	13.04	11.39	6.73	10.39	3.27	10.19
	40 mg	0	0	0	0	0.00	7.64	6.53	12.27	8.81	3.04	8.81
	40 mg	0	0.15	0	0.05	0.09	6.78	3.33	3.1	4.40	2.06	4.35
	40 mg	0	0.15	0	0.05	0.09	6.69	3.19	4	4.63	1.83	4.58
	40 mg	0	0	0	0.00	0.00	4.11	2	6.45	4.19	2.23	4.19
	40 mg	0	0	0	0.00	0.00	4.46	2.36	4.79	3.87	1.32	3.87
	40 mg	0	0.94	0	0.31	0.54	0.85	2.68	2.42	1.98	0.99	1.67
(b) (6)	80 mg	0.46	0	0.64	0.37	0.33	0.84	0.47	0.74	0.68	0.19	0.32
	80 mg	0.56	0	0	0.19	0.32	1.43	4.56	3.52	3.17	1.59	2.98
	80 mg	0.46	0	0.64	0.37	0.33	1.78	3.72	1.02	2.17	1.39	1.81
	80 mg	0	0	0.04	0.01	0.02	5.2	10.76	8	7.99	2.78	7.97
	80 mg	0.56	0	0	0.19	0.32	1.83	9.97	5.71	5.84	4.07	5.65
	80 mg	0.53	4.71	0.95	2.06	2.30	1.51	9.46	2.23	4.40	4.40	2.34
	80 mg	0.57	0	0	0.19	0.33	2.11	0.25	2.7	1.69	1.28	1.50
	80 mg	0	0	0.05	0.02	0.03	0.43	8.56	1.58	3.52	4.40	3.51

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**B: Normalization by Myosin**

Subject	Dose	Baseline 1	Baseline 2	Baseline 3	Baseline Average	Baseline SD	Wk 25 1	Wk 25 2	Wk 25 3	Wk 25 average	Wk 25 SD	Change from baseline
(b) (6)	40 mg	0	0.56	0	0.19	0.32	3.31	7.68	13.96	8.32	5.35	8.13
	40 mg	0.08	0.68	0	0.25	0.37	11.71	12.68	6.45	10.28	3.35	10.03
	40 mg	0.97	0	0	0.32	0.56	8.12	1.76	5.24	5.04	3.18	4.72
	40 mg	0.09	0.18	0	0.09	0.09	5.87	3.71	3.36	4.31	1.36	4.22
	40 mg	0.33	0.19	0.05	0.19	0.14	5.98	4.84	4.94	5.25	0.63	5.06
	40 mg	0.82	0	0	0.27	0.47	4.29	1.49	7.6	4.46	3.06	4.19
	40 mg	0.82	0.01	0	0.28	0.47	5.56	1.63	6.82	4.67	2.71	4.39
	40 mg	0.09	1.15	0	0.41	0.64	1.7	2.84	4.99	3.18	1.67	2.76
(b) (6)	80 mg	0.63	0.15	0.59	0.46	0.27	1.47	0.99	0.97	1.14	0.28	0.69
	80 mg	0.65	0	0.54	0.40	0.35	2.45	4.9	4.55	3.97	1.33	3.57
	80 mg	0.64	0.13	0.6	0.46	0.28	3.83	2.96	2.12	2.97	0.86	2.51
	80 mg	0	0	0.27	0.09	0.16	10.6	11.73	8.88	10.40	1.44	10.31
	80 mg	0.66	0	0.87	0.51	0.45	5.84	16.63	20.79	14.42	7.72	13.91
	80 mg	2.23	3.91	1.69	2.61	1.16	4.62	10.84	6.74	7.40	3.16	4.79
	80 mg	0.66	0	0.62	0.43	0.37	2.07	1.71	5.39	3.06	2.03	2.63
	80 mg	0	0	0.28	0.09	0.16	1.94	7.47	2.81	4.07	2.97	3.98

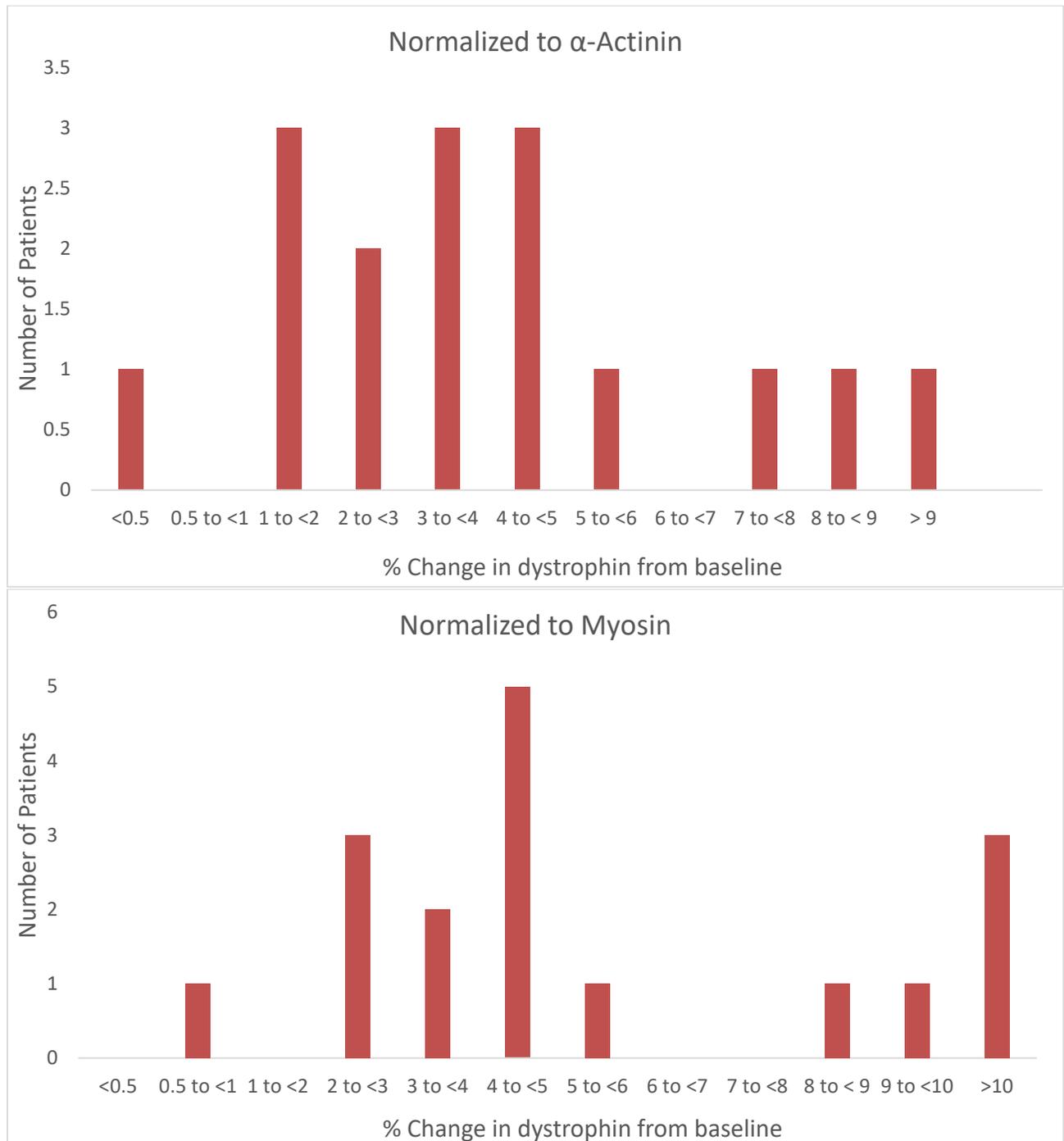
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The distribution of the amount of dystrophin at Week 24 at either dose is shown in Figure 5. Majority of the subjects had dystrophin between 1-5 % of normal.

**Figure 5 Distribution of Dystrophin (% of normal) at Week 24 at any dose**



Source: Primary Reviewer analysis

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**Statistical Reviewer’s Comments and Analyses (Dr. Xiang Ling)**

The data demonstrated statistically significant increase in dystrophin protein after 20-24 weeks of viltolarsen treatment at doses 40 and 80 mg/kg/wk (Table 7; see also Table 5 ). The individual patient’s percentage of normal dystrophin protein as measured by Western blot normalized to myosin is shown in Figure 6. The primary analyses are the paired t-tests within each dose level for the within-patient change in dystrophin protein measured by Western blot. There were 4 primary analyses as Western blot analysis included normalization to both myosin heavy chain and  $\alpha$ -actinin and there were 2 doses. Although a formal multiple testing procedure was not planned, the analysis results remain statistically significant for any reasonable adjustments for multiplicity control.

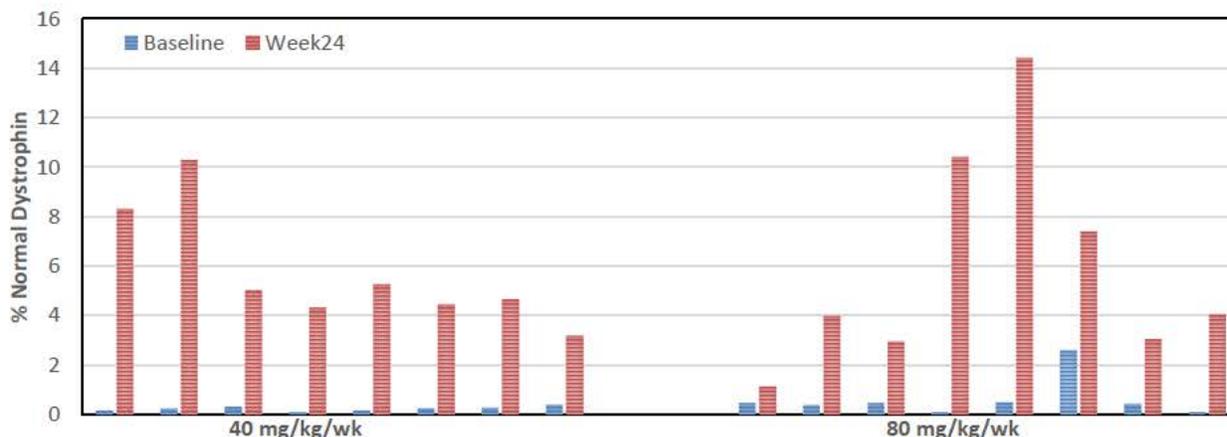
**Table 7 Analysis of Dystrophin by Western Blot**

	Normalized to Myosin (% of Normal)		Normalized to $\alpha$ -Actinin (% of Normal)	
	40 mg/kg/wk (N=8)	80 mg/kg/wk (N=8)	40 mg/kg/wk (N=8)	80 mg/kg/wk (N=8)
Baseline Mean (SD)	0.3 (0.1)	0.6 (0.8)	0.2 (0.2)	0.4 (0.7)
Week 25 Mean (SD)	5.7 (2.4)	5.9 (4.5)	5.4 (2.8)	3.7 (2.4)
Change from Baseline Mean (SD)	5.4 (2.4)	5.3 (4.5)	5.2 (2.8)	3.3 (2.5)
95% CI	(3.4, 7.4)	(1.6, 9.0)	(2.8,7.6)	(1.2, 5.3)
P-value (Paired T-Test)	0.0004	0.0123	0.0012	0.0074

CI=confidence interval; SD=standard deviation

Source: Statistics Reviewer analysis

**Figure 6 Individual Patient Percentage of Normal Dystrophin Protein Expression as Determined by Western Blot Normalized to Myosin**



Source: Statistics Reviewer analysis

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The reviewer also conducted a sensitivity analysis using a sign test, which is a nonparametric test with very few assumptions. The analysis showed similar results (nominal  $p = 0.0078$  for both doses; results not shown in table), supporting the primary analyses.

### Data Quality and Integrity for primary endpoint

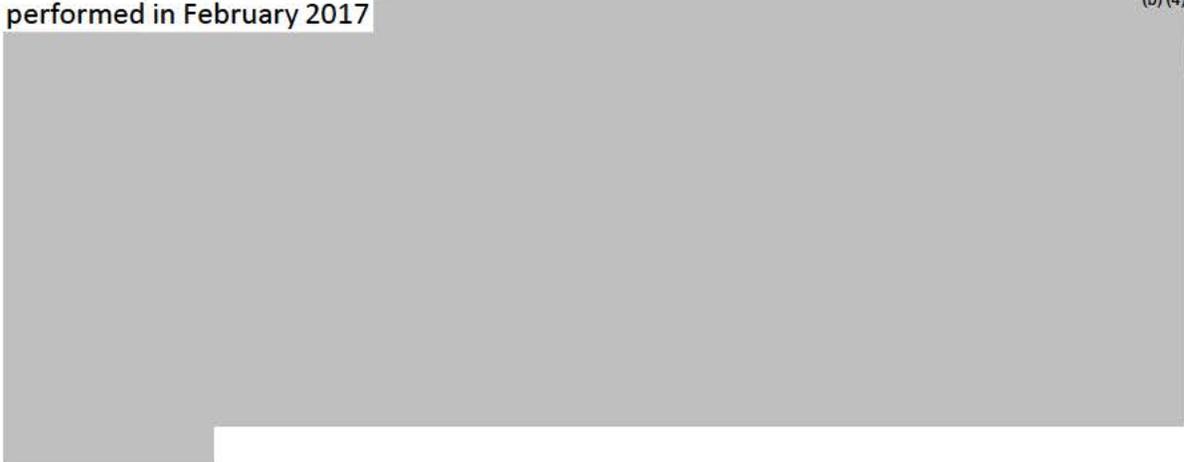
In this section, the assay methodology for the Western Blot analyses and its adequacy will be discussed.

#### **Western Blot Method Validation [OBP Reviewers-Dr. B. Aryal and Dr. A. Rao (Lead)]**

All dystrophin protein measures with western blot analyses were normalized to two different loading controls; (i) Coomassie-stained **myosin heavy chain** from post-transfer gels and (ii)  **$\alpha$ -actinin** immunostained in the same nitrocellulose membrane as dystrophin.

The applicant conducted 3 validations for the western blot methodology as discussed below:

- The first western blot method validation (Validation report: WB-NS-065/NCNP-01-201) was performed in February 2017 (b) (4)



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(b) (4)

The applicant attempted validation in multiple stages with slight modifications at each re-validation. There were minor failures in the first validation study but, overall, the applicant concluded that the western blot validation study was successful meeting most of the critical specified acceptance criteria.

- The applicant performed re-validation of western blot method

(b) (4)

. Overall, the validation was not successful because accuracy, spike/recovery, repeatability, intermediate precision, and limit of quantitation did not meet the predefined acceptance criteria.

(b) (4)

The re-validation study was failed,

(b) (4)

Therefore, a comment was communicated to the applicant requesting them to provide a validation study data to support the standard curve that was used for clinical sample analysis.

- The applicant then conducted a third re-validation study (Re-validation Report:010-MVR-081) using a five-points standard curve **0%, 1%, 3%, 10%, and 25% dystrophin** to align with the standard curve utilized in analyzing clinical samples and provided validation study report on 12/12/2019. Based on the information provided by the applicant there were no changes in DMD sample, normal controls, antibodies, and western blot method from the previous validation studies except for the % dystrophin used in the standard curve samples as discussed above. The applicant used the following assay acceptance /rejection criteria.

### Acceptance and rejection criteria

The applicant provided the following criteria for validation parameters to be considered as acceptable:

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- The limit of quantitation should not exceed 40% of the CV and the accuracy within 70% of the nominal concentration
- Standard curve must show an R<sup>2</sup> value of ≥95%
- Band intensity of 1% normal control is higher than DMD-only lane (0% dystrophin lane)
- Alpha actinin and myosin heavy chain loading controls are less than 50% of the relative standard deviation of average.
- If the discordant data are obtained (>30% of the determined values between two analysts), the analysis is considered a failure and would be repeated.

There were total of six gels run independently by two analysts (three gels per analyst). Each gel contained standard curve samples (0%, 1%, 3%, 10%, and 25% dystrophin) and quality control samples (1%, 5%, 10% and 20% dystrophin) to determine assay validity. The mean concentration of dystrophin at each QC level normalized by myosin heavy chain and alpha-actinin were calculated for each analyst separately from three gels. The applicant also calculated %CV and % accuracy for each analyst across 3 gels. The dystrophin normalized by myosin heavy chain passed all acceptance/rejection criteria but alpha-actinin normalization did not pass all criteria. The applicant has summarized their results in the following Table 8.

**Table 8: Western blot method validation results**

Acceptance and Rejection criteria	Derived % of Dystrophin normalized to myosin heavy chain	Derived % of Dystrophin normalized to alpha actinin
Standard curves must show an R <sup>2</sup> value of ≥0.95	Passed for both analysts 1 and 2 (R <sup>2</sup> value between 0.9963 - 1.0000)	Passed for both analysts 1 and 2 (R <sup>2</sup> value between 0.9949 - 0.999)
1% normal control is higher than DMD-only lane (0% Dystrophin lane)	Passed for both analysts 1 and 2	Passed for both analysts 1 and 2
Loading controls are less than 50% of the relative standard deviation of average	Passed for both analysts 1 and 2 (RSD between 1.9% - 9.1%)	Passed for both analysts 1 and 2 (RSD between 5.0% - 45.9%)
The limit of quantitation should not exceed 40% of the CV	Passed for both analysts 1 and 2	Failed for both analysts Analyst 1: 19.75% - 41.69% Analyst 2: 25.25% - 55.20%
The accuracy within 70% of the nominal concentration	Passed for both analysts 1 and 2	Passed for analyst 2 1% QC level failed by 3% for analyst 1

Source: Table 8 of western blot method validation report (Document ID 010-MVR-081)

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The mean values of % dystrophin obtained between two analysts using the same housekeeping protein for all QC samples were within 30% for all measures normalized to myosin heavy chain. For alpha-actinin normalization, the mean values of all QC samples were within 30% except for the 1% QC sample which showed a 50.7% difference between the two analysts.

The applicant also compared the validation data between original validation report WB-NS-065/NCNP-01-201 conducted in May 2017 and the current validation study. The validation data were comparable between two validation studies. Overall, myosin heavy chain normalization showed more consistent results compared to alpha-actin. Based on the nature of western blot method variability observed during validation, the applicant proposed to use both alpha-actinin and myosin heavy chain for normalization as orthogonal approaches to report clinical study sample data.

### Analysis of clinical samples with western blot

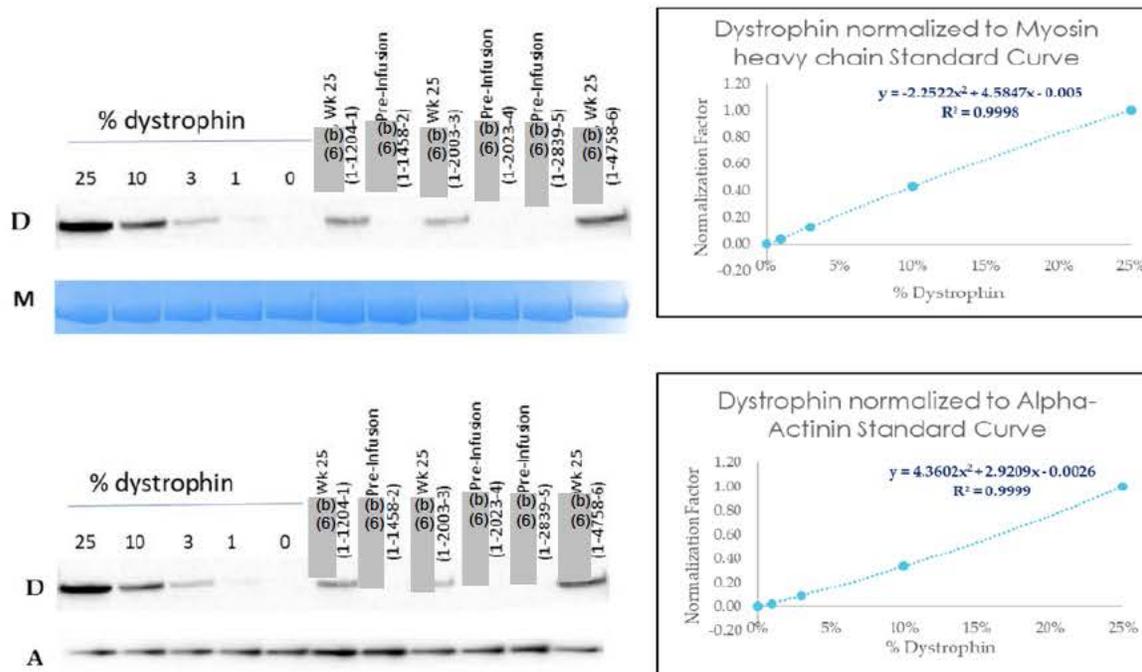
A total of 18 gels (9 gels from each cohort) were run to accommodate all 16 samples running in triplicate for each cohort. Each gel contained a molecular weight marker, 5 standard points for standard curve (0%, 1%, 3%, 10%, 25% dystrophin), and 6 clinical samples (3 patient samples, pre and post treatment). Equal amount (50 µg) of protein was loaded in each lane based on protein concentration determined by a BCA assay kit. Additionally, all gels were assessed with two normalization loading controls; alpha-actinin and myosin heavy chain to have band intensities within 50% of the relative standard deviation of average. As per the applicant, if discordant data were obtained (>30% of determined value) between two analysts, the analysis was viewed as a failure and would be repeated. All gels were assessed for meeting the pre-defined acceptance criteria as set in the protocol CL008SOP V4. Results were expressed as % dystrophin of normal. The representative images for blots, gel, and standard curves generated by normalization of dystrophin intensity with myosin heavy chain and alpha-actinin ran in the same gel during cohort 1 sample analysis is given below in Figure 8.

**Figure 8: A representative western blot and standard curves generated in the same blot using both alpha actinin and myosin heavy chain for normalization during clinical sample analysis**

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Source: Figure 4 western blot image data from Run 1, gel replicate 2 for cohort 1 page 47 of WB, IF, RT-PCR dystrophin bioanalytical report

**OBP Reviewer's comment:** The normal control used in this study can be considered representative of dystrophin level in healthy individuals because it was prepared from 5 non-DMD tissues. Although normalization with myosin heavy chain appears to be a more reliable loading control than alpha-actinin and passed all of the applicant's acceptance criteria, overall validation study data demonstrated that there is high variability in the western blot method. The % CV for dystrophin normalized to myosin heavy chain was in the range of 5.01-35.09 % and % CV for dystrophin normalized to alpha-actinin was in the range of 19.75 – 55.2 % across three gels for QC samples. Similarly, % accuracy of dystrophin normalized to myosin heavy chain was in the range of 2.4 -35.9 % and % accuracy of dystrophin normalized to alpha-actinin was in the range of 24.2 – 61.83 % across three gels for QC samples.

A validation study was performed using a limited number of samples (4 QC samples repeated in 3 gels per analyst) but during clinical sample analysis, a total of 32 samples from cohort 1 and cohort 2 were run in triplicate in 18 gels. The high variability in % dystrophin was also observed during clinical sample analysis as indicated by %CV among three gels for each sample as shown in Tables 10-15 of study-201 report 010-CSR-049 (Table not included in this memo). The % CV for dystrophin in Wk25 samples normalized by myosin heavy chain was in the range of 12-73% whereas % CV for dystrophin in a Wk25 samples normalized by alpha-actinin was even higher, 28-124%, across all samples. This variability is likely inherent to the western blotting method itself. The sponsor's use of mass spectrometry as an orthogonal method supports the relative quantitation reported by the western blotting method.

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*I (BA/AR) looked at the full-length gel images used for method validation and clinical sample analysis. Gel images from clinical sample analysis show a distinct band at expected molecular weight of intact dystrophin protein for Wk25 samples, whereas no visible bands are present in most pre-infusion samples except for # (b) (6)*

*Overall, the western blot method was validated using myosin heavy chain and alpha actinin as housekeeping proteins for normalization. There is some residual variability in dystrophin results in this method using either normalization control proteins, but the degree of variability was less when dystrophin intensities were normalized with myosin heavy chain compared to alpha-actinin. Therefore, dystrophin protein results obtained by myosin heavy chain normalization may be relatively more reliable than alpha-actinin normalization.*

### **Efficacy Results – Secondary and other relevant endpoints**

Secondary endpoints discussed below are not included in formal statistical testing procedure, therefore all reported p-values are nominal p-values. For each visit where a biopsy was performed, up to 3 planned responses for each test were averaged to attain a single result for summarizing and analyzing.

#### **Dystrophin by Mass Spectrometry (MS):**

Dystrophin quantification by MS methodology was adequately validated, as discussed under the 'Method validation by MS' subsequent section. The quantification of dystrophin by MS can be supportive of the western blot results. MS analysis on muscle homogenate protein extracts showed that viltolarsen resulted in higher levels of dystrophin protein after 24 weeks of treatment than at baseline, although the amounts were lower than that obtained by western blot analysis.

**Baseline Dystrophin:** Mean baseline dystrophin protein measured by MS was 0.5% and 0.6% of normal in the viltolarsen 40 and 80 mg/kg/wk groups, respectively (Table 9). (Note: These % dystrophin at baseline is similar to that obtained by western blot method, i.e. 0.3 and 0.6% of normal, when normalized against myosin).

**Post-treatment Dystrophin:** At Week 25, mean increases from baseline of 1.5% and 3.7% of normal levels were observed in the 40 and 80 mg/kg/wk groups, respectively. The median increase was 1.7 and 1.9%, respectively for the 40 and 80 mg/kg/wk doses. (Note: The post-treatment dystrophin levels are lower than that with western blot analysis, although baselines are similar with the two methods. The reason for this is unclear.)

The mean change from baseline for the viltolarsen 40 and 80 mg/kg/wk groups had nominal p-values of 0.0061 and 0.03, respectively. No statistically significant difference between the dose groups was identified for dystrophin products by MS (Table 9).

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**Table 9 Dystrophin Production by MS (mITT Population)**

Dystrophin (%) Visit/ Statistic	40 mg/kg/wk (N=8)		80 mg/kg/wk (N=8)	
	Obs	CFB	Obs	CFB
<b>Baseline</b>				
Mean (SD)	0.5 (0.15)	--	0.6 (0.19)	--
Median	0.6	--	0.6	--
Min, Max	0.2, 0.8	--	0.2, 0.9	--
<b>Week 25</b>				
Mean (SD)	2.1 (1.1)	1.5 (1.1)	4.2 (3.7)	3.7 (3.8)
Median	2.1	1.7	2.6	1.9
Min, Max	0.0, 3.3	-0.7, 2.7	1.3, 10.8	0.8, 10.5
95% CI	--	(0.6, 2.4)	--	(0.5, 6.8)
P-value* (Paired T-Test)	--	<b>0.0061</b>	--	<b>0.0300</b>
95% CI (80 mg – 40 mg)	--	--	--	(-1.08, 5.37)
P-value (2-Sample T-Test)	--	--	--	0.16

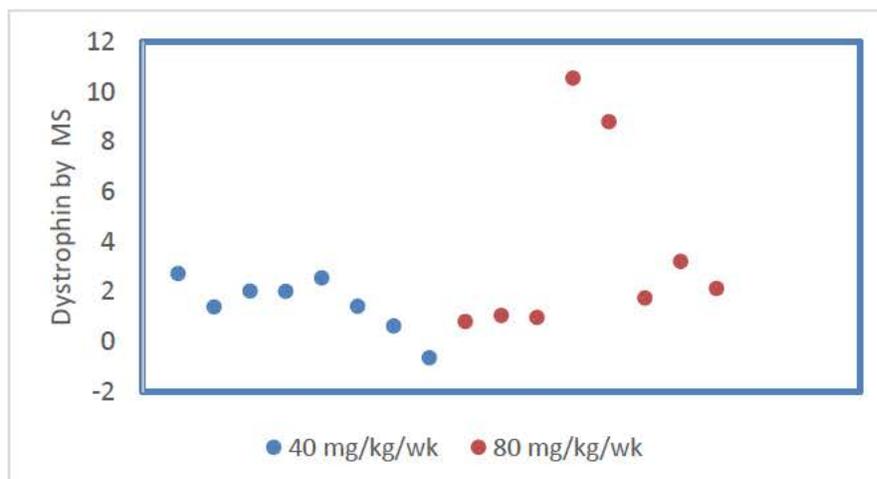
CFB=change from baseline; CI=confidence interval; Max=maximum; Min=minimum; Obs=observed; SD=standard deviation

\*Note: all p-values in the Table are nominal p-values

Source: verified by the reviewer

The amount of truncated dystrophin was similar between the two doses for 14 patients, except for 2 patients at the 80 mg/kg/week dose that showed higher amounts of dystrophin by MS methodology than the rest of the patients, as shown in Figure 9.

**Figure 9 Dystrophin by MS**



Source: Primary Reviewer analysis

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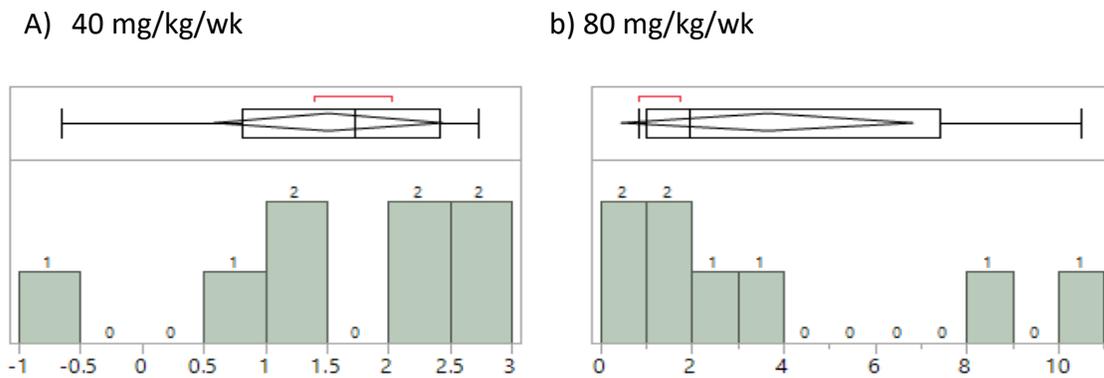
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**Primary Reviewer's Comment:** The 2 patients with higher amounts of truncated dystrophin at 80 mg/kg/wk in this figure were also the two patients that showed higher amounts on Western blot when normalized to myosin.

The distribution of % dystrophin by MS at 40 and 80 mg/kg/wk can be visually appreciated in Figure 10 as well.

**Figure 10 Distribution of % dystrophin by MS at 40 and 80 mg/kg/wk**



Source: Primary Reviewer analysis

**Mass spectrometry (MS) method validation [OBP Reviewers-Dr. B. Aryal and Dr. A. Rao (Lead)]**

For quantification of dystrophin levels in the clinical study samples using mass spectrometry, in-gel digested tryptic peptides were analyzed by LC-MS/MS ((validation report NoNSP-W6-779(R1)). After protein extraction from tissue biopsies, 50 µg of each tissue extract spiked with or without 25 µg of SILAC- (stable isotope labelling by amino acid in cell culture) labeled myotube extract was loaded in the gel. A standard curve comprised of **0%, 1%, 3%, 10%, and 25% of dystrophin levels** was generated using five non-DMD samples (considered 100% dystrophin) and 1 DMD sample (0% dystrophin) as described for the western blot method. The gel included standard curve samples, quality control samples (low 2%, medium 7% and high 15% dystrophin), a BL (DMD without SILAC spiked in) and a SL (SILAC labeled myotube alone) as shown in the figure below. A total of two gels (gel A and gel B) were run for method validation proposes.

MW	25%	10%	3%	1%	0%	BL	2%	7%	15%	SL	
1	2	3	4	5	6	7	8	9	10	11	12

All validation runs were evaluated against the target acceptance criteria specified in the following Table 10.

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**Table 10: Target acceptance criteria for MS method validation**

Parameters	Target Acceptance Criteria
Linearity	Curves should fit the linear regression type weighted 1/x or 1/x <sup>2</sup>
Precision and Accuracy	Inter-assay of 2 response curves %Nominal is 70.0% - 130.0%
Selectivity of instrument	Determined by absence of signal in mobile phase samples
Stability in injection medium (reconstitution solution)	%Nominal is 70.0% - 130.0% for stability samples, at all QC levels.
Carryover	At the RT of the IS, the response is ≤10.0% of the IS response of the LOQ. At the RT of the analyte, the response is ≤25.0% of the analyte response of the LOQ.
Limit of Quantitation (LOQ)	LOQ at 1% of normal dystrophin level based on feasibility study.

Source: Table 1 of MS fit-for-purpose validation plan NSP-W6-779 page 124

The system suitability of LC/MS/MS was performed using a six synthetic peptides mixture to evaluate system sensitivity, mass accuracy and LC retention time. Three selected dystrophin surrogate peptides (DYS\_1, DYS\_2 and DYS\_3] were initially used for quantification and two Filamin C peptides (FILC\_1 and FILC\_2) for background normalization.

The dystrophin protein level was calculated using peak ratios of dystrophin peptides normalized to Filamin C (internal control) peptides peak area ratio as follows:

$$\text{Dystrophin protein level} = \frac{\text{Dystrophin ratio}}{\text{Filamin C ratio}} = \frac{\frac{\text{Dyst peptide}}{\text{Dyst SIL peptide (IS)}}}{\frac{\text{FilC peptide}}{\text{FilC SIL peptide (IS)}}}$$

The applicant provided a list of deviations in their original validation plan, as discussed below:

- The most notable deviation was the exclusion of precision parameter in the QC samples during method validation due to limited QC samples (one replicate of QC samples at each level for a total of two gels).
- The DYST\_3 was found to be least sensitivity and out of specification in accuracy at low QC level (>30% difference in concentration from the nominal value) and some calibrants during their validation study. Therefore, data were processed using mean of DYST\_1 and DYST\_2 peptides normalized to mean value of filamin C peptides with a linear weighted 1/x regression fit and subtraction of 0% standard. Other reported deviations were minor and are not expected to have a significant impact in the method validation.

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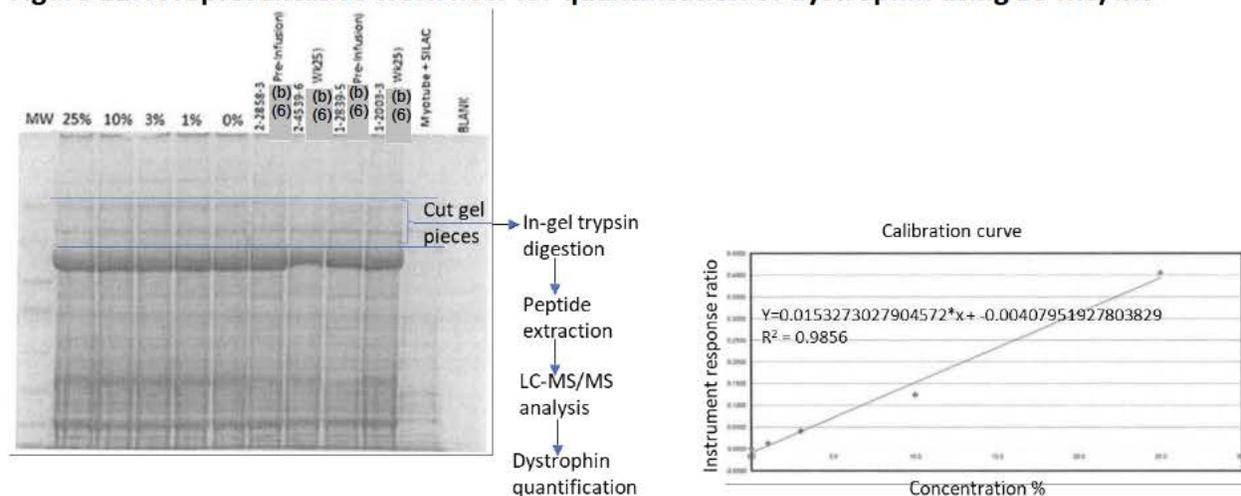
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All validation parameters were evaluated using mean of two dystrophin peptides (DYST\_1 and DYST\_2) or only DYST\_2 peptide normalized to mean of two FILC peptides ratios. The applicant did not analyze data for precision as per their documented deviation during validation.

Overall, their validation data demonstrates that LC-MS/MS method validation met the acceptance criteria with respect to linearity, specificity, sensitivity, accuracy, and stability within a theoretical concentration range of 1.0% to 25% for dystrophin.

**Analysis of clinical samples by MS:** For clinical sample analysis, sample preparation is the same as method validation. Dystrophin peptides were identified and quantified with LC MS/MS detection. The concentrations were calculated using peak area ratios of the corresponding dystrophin peptide product ions normalized to peak area ratios of filamin C peptides product ions as previously described in an equation under MS method validation section. Method validation was performed with and without subtraction of the 0% Standard but since the level of background dystrophin is unknown in clinical samples, clinical samples were analyzed without subtraction of 0% Standard. A MS analysis work flow generated by the reviewer using information from the application is given in Figure 11.

**Figure 11: A representative work flow for quantification of dystrophin using LC-MS/MS**



Source: MS dystrophin Bioanalytical report, Figure 6 Coomassie blue stained gel 1 (A) on page 51, and corresponding calibration curve taken from page 5 of Revised raw data-study 201 MS dystrophin images.

For initial analysis of assay validation and clinical study sample, data were analyzed using average value of both DYST\_1, DYST\_2 and their internal standards (DYST\_1\_IS and DYST\_2\_IS). The validation was performed using limited samples from only 2 gels but during clinical sample analysis, DYST\_1 and DYST\_1\_IS responses were found to be significantly lower than expected or not detected in several clinical samples and calibrants while DYST\_2 and FILC\_1 and FILC\_2 peptides had acceptable responses during sample analysis. Therefore, data was reprocessed using only DYST\_2 ratio normalized with the mean of both FILC\_1 and FILC\_2 ratios. The

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applicant states that original validation data was re-processed using DYST\_2 ratio normalized to mean of FILC ratios to verify that quantitation of dystrophin using only one peptide as an alternative is reliable and accurate.

Extended validation study of the carryover parameter indicates that some carryover was always observed in the first analyte-free injection following QC sample injection regardless of QC concentrations, but carryover was insignificant or absent in the second analyte-free sample injection. Therefore, during clinical sample analysis, the calibrant and QC samples were injected in the order of increasing concentration followed by one analyte-free sample (BL or SL) in between pre-and post-dose samples as shown in the following Table provided by the Sponsor. System suitability injections were performed before and after the acquisition sequence as listed in the following Table 11. A total of 32 clinical samples with each sample in duplicate gels (gel A and Gel B) were analyzed using this method. The Sponsor did not report results from 4 clinical samples from gel B due to low response of DYST\_2 peptide.

**Table 11 : Acquisition sequence for study sample analysis by LC-MS/MS**

Sample
Analyte-free sample_1
Analyte-free sample_2
BL
SL
STD0
STD1
STD3
STD10
STD25
Analyte-free sample_3
Sample 1
Analyte-free sample_4
Sample 2
Analyte-free sample_5
Sample 3
Analyte-free sample_6
Sample 4

Analyte-free sample: BL or SL.

Source: Table 24 of MS dystrophin bioanalytical report -study 201 (NSP-P2-419)

*OBP Reviewer's comment: Method validation was performed for two of the three dystrophin peptides but during sample analysis only one dystrophin peptide (DYST\_2,) was found to be sensitive enough to quantify dystrophin. Based on their validation data for calibration curve performance, results are comparable using either DYST\_2 or DYST\_1 and DYST\_2. It should be*

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*noted that clinical sample analysis results would have been more reliable if data were obtained by analyzing multiple DYST peptides instead of relying on one peptide.*

*The applicant did not analyze method precision during validation but based on the back-calculated concentrations of dystrophin calibrants at each concentration levels from all standard curves used during clinical sample analysis (n=14), the % CV was <15%. This indirectly demonstrates that MS method has acceptable precision.*

*MS method seems to be reliable technique to accurately measure dystrophin content in the gel-extract of clinical samples because (i) it relies on quantification of dystrophin specific peptides (ii) dystrophin peptides are normalized with filamin C peptide and (ii) both dystrophin and filamin C peptides are normalized with their own SILAC labeled standards peptides.*

*The applicant used the same standard curve (0, 1, 3, 10, 25% dystrophin) for both western blot and MS analysis; however, based on the nature of the assays there could be some variability in the results obtained from these two methods. The western blot analysis is based on the relative quantitation after detection of a single intact dystrophin band at around 427 kDa but in MS analysis, dystrophin peptides are analyzed from a gel piece containing multiple proteins with molecular masses ranging from 260 to 460 kDa. Therefore, MS analysis may include full length dystrophin protein (427 kDa band) and potentially degraded dystrophin protein fragment with molecular weight >260 kDa. The detection methods and relative quantitation approaches for the western blotting and MS methods are inherently different and could contribute to the differences in relative quantitation levels reported by each method.*

*Overall, based on the current validation approach, MS data can be used to support the western blot data. If further validated using multiple dystrophin peptides that are sensitive to ionization source in MS and have high in-gel extraction efficiency, the applicant's MS method could provide superior quantitative results and be even more reliable than western blot results at comparable ranges of target dystrophin.*

### **Dystrophin by Immunofluorescence (IF):**

The applicant measured both the **dystrophin positive fibers** and the **dystrophin intensity** by IF. However, the dystrophin intensity assessments are unreliable as discussed under 'Method validation by IF'. Therefore, I (VT) only present results on dystrophin positive fibers from the IF analysis of the muscle biopsy. Appropriate localization of dystrophin at the plasma membrane was confirmed by co-staining the biopsies for dystrophin and alpha-sarcoglycan. Alpha-sarcoglycan is a dystrophin associated protein that co-localizes with dystrophin at the myofiber plasma membrane but shows stronger immunostaining on dystrophin-positive vs. dystrophin-negative myofibers (see Method Validation by IF in the following section for details).

The dystrophin positive myofibers were higher after 24 weeks of treatment compared with baseline in all patients.

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**Baseline dystrophin-positive myofibers:** Mean baseline dystrophin-positive myofibers measured by IF was 1.5% and 1.8% of BMD (i.e., 80% of normal dystrophin) in the viltolarsen 40 and 80 mg/kg/wk groups, respectively (Table 12).

**Post treatment dystrophin-positive myofibers:** At Week 25, mean increases from baseline in dystrophin-positive myofibers were observed: increases of 12.8% and 33.0% in the 40 and 80 mg/kg/wk groups, respectively. The median increases from baseline were 11.3 and 26.9% for the 40 and 80 mg/kg/wk groups, respectively.

The mean change from baseline in the percent dystrophin positive myofibers with viltolarsen 40 and 80 mg/kg/wk groups had nominal p-values of 0.0028 and 0.0026 respectively. According to the method validation review by Drs. Aryal and Rao, the immunofluorescence data is only recommended to be used qualitatively for the co-localization but may not be appropriate to use as quantitative measures to make any conclusion for dose or efficacy (see Method validation section of the review).

**Table 12 Percent Dystrophin-positive Myofibers by IF (mITT Population)**

Dystrophin-positive Fibers (%) Visit/ Statistic	40 mg/kg/wk (N=8)		80 mg/kg/wk (N=8)	
	Obs	CFB	Obs	CFB
<b>Baseline</b>				
Mean (SD)	1.5 (1.0)	--	1.8 (2.4)	--
Median	1.8	--	0.9	--
Min, Max	0.2, 2.5	--	0.1, 6.8	--
<b>Week 25</b>				
Mean (SD)	14.2 (7.8)	12.8 (8.1)	34.8 (20.4)	33.0 (20.4)
Median	13.1	11.3	27.9	26.9
Min, Max	6.2, 32.2	4.0, 31.5	15.5, 72.2	8.8, 68.1
95% CI	--	(6.0, 19.5)	--	(15.9, 50.1)
P-value (Paired T-Test)	--	<b>0.003</b>	--	<b>0.003</b>
95% CI (80 mg – 40 mg)	--	--	--	(2.7, 37.7)
P-value (2-Sample T-Test)	--	--	--	0.03

CFB=change from baseline; CI=confidence interval; Max=maximum; Min=minimum; Obs=observed; SD=standard deviation

\*Note: all p-values in the Table are nominal p-values

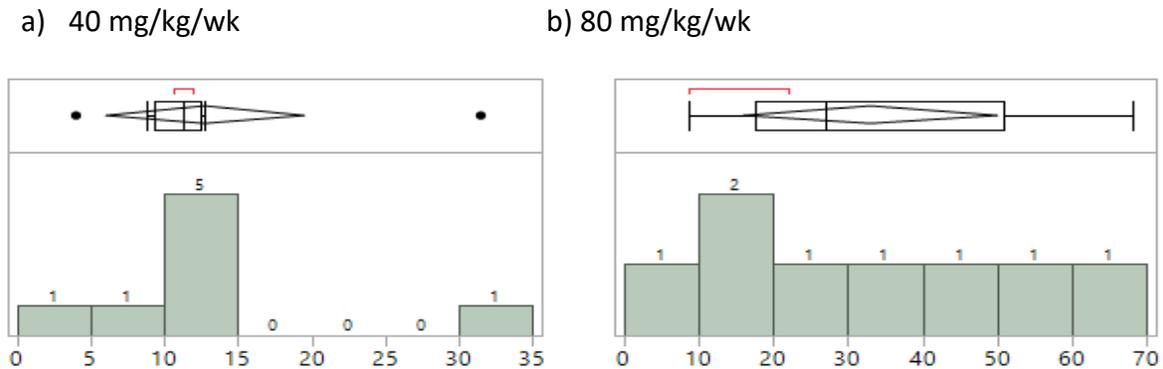
Source: verified by the Reviewer

There appeared to be a dose related increase in dystrophin positive fiber. The frequency distribution of dystrophin positive fibers at the 40 and 80 mg/kg/week doses are shown in Figure 12.

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**Figure 12 Distribution of dystrophin positive fibers by IF at 40 and 80 mg/kg/wk**



Source: Primary Reviewer's Analysis

Note: The X-axis is different in Figure 12 a and b

### **Method validation for Immunofluorescence (IF) [OBP Reviewers-Dr. B. Aryal and Dr. A. Rao (Lead)]**

Immunofluorescence (IF) staining was performed using standard operating procedure CL006SOP. Prior to dystrophin staining of muscle biopsies, Hematoxylin and Eosin staining (H&E staining) of parallel muscle sections of all samples were performed with the following acceptance criteria:

1. <10% of myofibers can show evidence of freeze artifact.
2. >80% of myofibers must be visible in cross section.
3. >50% of the muscle section area must be comprised of myofibers.
4. The analytical samples will only be examined if they contain greater than 200 muscle fibers per section.

The IF staining was performed only if the H&E staining met all 4 acceptance criteria above. The proportion of dystrophin-positive myofibers was determined by co-staining of tissue sections with dystrophin and laminin alpha 2 (merosin). Appropriate localization of dystrophin at the plasma membrane was confirmed by co-staining for dystrophin and alpha-sarcoglycan. The following primary and secondary antibodies were used in IF staining; Anti-dystrophin antibody (ab15277), Anti-laminin alpha 2 (LAMA2) antibody (MAB1922), Anti- $\alpha$ -sarcoglycan (SGCA) antibody (IVD3(1)A9), Alexa Flour<sup>®</sup> 594 AffiniPure goat anti-rabbit IgG, and F(ab')<sub>2</sub> fragment specific (111-585-006), Alexa Flour<sup>®</sup> 488 Goat anti-mouse IgG1 (A21121). The stained slides are imaged using Leica Aperio Versa 8 scanner. The IF images are analyzed using imagescope software for the following measures:

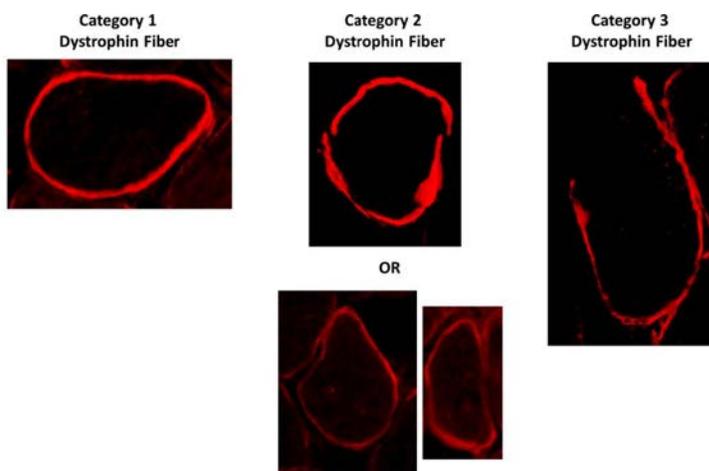
- Number of dystrophin positive fibers
- Total number of LAMA2 positive fibers
- Percent dystrophin positive fibers
- Intensity of dystrophin and LAMA2 staining
- Intensity of dystrophin and SGCA2 staining

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**Quantification of dystrophin positive fibers** is broken down into three categories based on the physical integrity of plasma membrane and immunostaining intensity: (1) complete (100%) dystrophin positive fibers with no breaks and strong immunostaining, (2) dystrophin positive fibers with partial membrane break, and (3) incomplete plasma membrane staining or both and dystrophin positive fibers with major break ( $\leq 50\%$ ). The applicant listed all fibers meeting these categories as dystrophin positive fibers. If a membrane break is  $>50\%$  it is considered a negative fiber. **Quantification of LAMA2 positive fibers** follows the similar procedure. Representative images of all three categories are given in the following Figure 13 provided by the applicant.

**Figure 13: Quantification of dystrophin positive fibers using ImageScope software**



Source: Figure 1, page 94 of immunofluorescence method validation and protocol for dystrophin protein

The **percent dystrophin positive fibers** are calculated as  $[\text{number dystrophin positive fibers}/\text{number of LAMA2 positive fibers}] * 100$ . Gain threshold is set using a BMD internal control; the % dystrophin positive fibers in BMD samples are approximately 80%. The minimum and maximum threshold intensity are set as 0.2 and 1.0 respectively as default values. The intensity of dystrophin staining is normalized to the intensity of laminin alpha 2 to calculate the quantity of dystrophin-based intensity. The applicant states that the positive colocalization of dystrophin and  $\alpha$ -sarcoglycan is confirmed only if 100% overlap occurs between two stains in the composite image.

Based on the information provided by the applicant, the IF method was initially developed as a qualitative method (IF-NS-065/NCNP-01-201) to demonstrate co-localization of dystrophin with myofiber control proteins but with Agency's recommendation the method validation was improved for quantification of dystrophin. The IF method validation was performed using following samples.

1. DMD patient without revertant fibers (ID# (b) (6)); age: (b) (6) years old) – 2 slides, each containing 3 tissue sections

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2. DMD patient with revertant fibers (ID# (b) (6) age: (b) (6) years old) – 2 slides, each containing 3 tissue sections
3. Non-DMD patient (ID# (b) (6); age (b) (6) years old) – 2 slides, each containing 3 tissue sections
4. Manifesting female carrier patients (ID# (b) (6) (age: (b) (6) years old), (b) (6) (age: (b) (6) years old), (b) (6) (age: (b) (6) years old)) – 6 slides, each containing 3 tissue sections

The non-DMD and DMD samples with and without revertant fibers were used as controls for the staining. The manifesting carrier samples were used as test samples. The validation study was performed using three (b) (4) SOPs; (i) CL006SOP-dystrophin staining for exon skipping, (ii) CL011SOP-H&E staining for exon skipping, and (iii) CL010SOP-quantitation of IF images in muscle biopsies. Samples were stained and analyzed with 3 replicates by 2 analysts for the presence of revertant muscle fibers, the % dystrophin positive muscle fibers and relative quantities of dystrophin based on the intensity. All sections should pass all acceptance criteria for H&E staining before they could be used for IF validation study. If the sample does not meet the acceptance criteria or the freezing artifacts were observed, the secondary samples sectioned from the corresponding tissue biopsy were used.

In addition to H&E staining, immunostaining of samples met the following acceptance criteria:

1. The normal muscle biopsy must show strong and continuous immunostaining and co-localization of dystrophin and laminin alpha 2 proteins. All myofibers must show strong and continuous immunostaining and co-localization of dystrophin and  $\alpha$ -sarcoglycan proteins.
2. The DMD control muscle biopsy with no revertant fibers must show negative dystrophin staining, faint  $\alpha$ -sarcoglycan staining and normal continuous and high-level laminin alpha 2 immunostaining.
3. The DMD with revertant fibers and manifesting carriers should show a mosaic pattern of dystrophin positive and dystrophin-negative fibers with strong and continuous laminin alpha 2 immunostaining. Similarly, dystrophin-positive fibers must show strong immunostaining with  $\alpha$ -sarcoglycan, whereas dystrophin-negative myofibers show faint immunostaining with  $\alpha$ -sarcoglycan. The dystrophin positive fibers should show co-localization of dystrophin and  $\alpha$ -sarcoglycan.
4. All **positive controls** (non-DMD biopsy samples stained with primary dystrophin antibody) should show positive dystrophin staining and all **negative controls** (non-DMD biopsy samples stained in absence of primary dystrophin antibody) should show no positive staining of dystrophin. Analytical samples were examined only if positive and negative controls passed the IF staining procedure.

The results reported by the applicant demonstrate that all samples met the acceptance criteria. All 3 female carriers showed similar values for dystrophin and  $\alpha$ -sarcoglycan levels and these values were comparable between analyst 1 and analyst 2. The differences in CV% between two analysts for determination of dystrophin positive fiber counts and

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intensity for dystrophin normalized to either laminin alpha-2 or SGCA for the manifesting carriers were in the range of 5.4 -7.2%. Non-DMD samples showed positive staining of dystrophin, laminin alpha 2, and SGCA in all muscle fibers. The specificity of antibodies were demonstrated with no dystrophin staining in the DMD samples without revertant fibers and positive staining for laminin alpha-2 in all muscle fibers.

*OBP Reviewer's comment: All pre-defined validation acceptance criteria were met but the overall method and the method's validation approach was qualitative because all pre-defined acceptance criteria are subjective rather than more objective or quantitative for precision/repeatability between two analysts. They calculated % CV for dystrophin positive fiber count and intensity for both analysts, which were within reasonable numbers, but there were no predefined acceptance criteria for precision or repeatability.*

**IF Method Bridging Study:** Following method validation, an IF bridging study was conducted to evaluate the exposure time setting by benchmarking against BMD samples for IF staining and quantitation of dystrophin in the clinical samples. The main objective of the bridging study was to determine appropriate gain across all test samples so that BMD samples showed approximately 80% dystrophin positive fibers, which is expected in BMD samples. This gain was used across all tested samples for the analysis. One analyst was involved in both slide staining and analysis. The staining method and dystrophin positive fiber analysis were same for original validation and bridging study (CL006SOP).

There were no acceptance criteria but based on the applicant's response to our information request, staining is considered successful if the defined approach is able measure 100% dystrophin fibers in non-DMD control, dystrophin-positive myofibers of known revertant myofibers in a previously defined DMD sample and known partial dystrophin-positive myofibers in a previously defined BMD sample.

The applicant states that the default settings for image intensity thresholds (0.2 minimum and 1.0 maximum) was taken by both analysts during % dystrophin positive fiber count. The percent dystrophin-positive fibers were approximately 80% for the BMD, 100% for non-DMD, 11% for DMD from cohort 1 and 1% for DMD with revertant fibers. In DMD sections with revertant fibers and from cohort 1, all muscle fibers showed positive staining for laminin alpha 2 with a small proportion of dystrophin-positive muscle fibers. Colocalization of dystrophin and laminin alpha-2 were observed in all dystrophin-positive fibers. The colocalization of dystrophin and  $\alpha$ -sarcoglycan was observed in all muscle types with dystrophin to  $\alpha$ -sarcoglycan intensity ratio closer to 1 (0.778 to 1.2).

For **intensity measurement**, the applicant states that the intensity thresholds *were modified by each analyst* to achieve 100% dystrophin intensity around the plasma membrane of normal muscle fibers if the tuning window did not capture 100% dystrophin. This indicates that different threshold values may have been used across clinical samples during analysis which may raise the question for reliability of their IF data from intensity measurement.

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According to the applicant co-localization of dystrophin and  $\alpha$ -sarcoglycan was determined by taking independent digital images for the two individual proteins and merging them together. The positive localization is determined only if 100% overlap between the two stains occur in the composite image. The applicant only provided one representative image for dystrophin/ $\alpha$ -sarcoglycan or dystrophin/laminin alpha 2 in their report but did not provide co-localized images for all bridging study samples. For review purpose, the colocalization was evaluated by side-by-side comparison of a few randomly selected images.

### Analysis of Clinical samples

During sample analysis, two additional slides were included in each batch staining to verify staining consistency. One BMD slide was used to set the appropriate exposure time and gain setting so that the BMD sample shows 80% dystrophin positive fibers and one non-DMD slide as a positive control to show 100% dystrophin positive fibers. According to the applicant, most biopsies sectioned from primary biopsies in cohort 1 and cohort 2 passed all 4 sample acceptance criteria for H&E staining. For samples that failed to meet all 4 acceptance criteria, tissue sections from secondary biopsy (back-up biopsies) were used. One sample from cohort 1 ( (b) (6) week25, 3-4370-3) and one sample from cohort 2 (b) (6) pre-infusion, 1-6743-2) failed to pass the acceptance criteria even after re-testing with secondary biopsy samples; therefore, for both failed samples, the data obtained from primary biopsy samples were retained but identified as having failed the acceptance criteria. The CL011SOP allows for sectioning from a secondary biopsy if the primary biopsy failed to meet H&E staining.

From IF method validation report it was not clear about threshold setting during their clinical sample analysis. There were conflicting statements about the threshold setting. In response to our information request, the applicant clarified that to determine % dystrophin positive myofibers, a default setting of intensity threshold (*0.2 minimum and 1.0 maximum*) and constant gain was used during analysis of experimental samples. However, during intensity measurement, the intensity thresholds were modified by each analyst with a goal of achieving continuous staining around each positive myofiber for each immunostain. This resulted in uninterpretable intensity values because there were no predefined rejection/acceptance criteria associated with those modified values. The applicant acknowledges that sample analysis based on the % dystrophin intensity measurement is difficult to interpret and was not included in interpretation of drug-induced dystrophin expression.

*OBP Reviewer's comment: The applicant used a validated IF method to analyze clinical samples. The validation approach used by the applicant is more qualitative rather than quantitative in terms of intensity measurement because the acceptance criteria were subjective and there were no pre-defined quantitative acceptance criteria for accuracy and precision. I randomly checked the IF images in their submission for relative number of dystrophin positive fibers in pre-treatment and weeks 25 samples, the localization of dystrophin in the plasma membrane, co-localization of dystrophin and  $\alpha$ -sarcoglycan or laminin alpha 2 and any potential duplication of tissue section using alpha-laminin stained images. There were some images with high background staining for dystrophin (red) for both pre-infusion (e.g., subject # (b) (6) )*

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and Wk25 (e.g. subject # (b) (6)) samples but myofiber membrane staining was distinguishable from background staining. The background staining may have some impact in the intensity measurement, but minimum effect is expected in dystrophin positive fiber counts because fiber count is based on membrane staining of each myofiber. Based on my evaluation, I could not find any duplication of images or captured fields.

The co-localization of dystrophin with laminin alpha 2 or  $\alpha$ -sarcoglycan was not provided in the merged images but side-by-side comparison of dystrophin, laminin alpha 2 and  $\alpha$ -sarcoglycan stained images showed outer membrane localization of all three proteins.

Based on the image analysis approach disused above, measurement of percent dystrophin positive fibers (PDPF) is more reliable than intensity measurement. The plasma membrane staining-based PDPF measurement appears to show drug-induced changes in the dystrophin positive fibers but does not provide information about the amount of dystrophin protein present in each fiber or in the entire tissue section. All fibers are considered as dystrophin positive if the intensity level is above the threshold value (0.2 minimum and 1.0 maximum) and this measurement does not discriminate fibers based on intensity levels. The Applicant is not using intensity data to make any conclusion, therefore, without inclusion of intensity data and demonstration of good correlation between PDPF and intensity measurement, immunofluorescence data is only recommended to be used qualitatively for the co-localization but may not be appropriate to use as quantitative measures to make any conclusion for dose or efficacy.

### **Dystrophin RNA by reverse transcription polymerase chain reaction (RT-PCR) (Percent exon skipping):**

The proposed mechanism of action of viltolarsen is to bind to exon 53 region of precursor mRNA of dystrophin and block the inclusion of exon 53 in the mature mRNA transcript. The RT-PCR assay was used to provide evidences that viltolarsen is capable of binding to its target to produce in-frame mRNA over the skipped region that can produce “Becker-like” dystrophin protein.

RT-PCR on patient muscle biopsy mRNA using primers flanking each patient’s deletion neighboring exon 53 provides this direct means of assessing whether exon 53 is excluded from the patient’s dystrophin mRNA. The extent of exclusion is a measure of viltolarsen effectively binding to a patient’s dystrophin mRNA.

Baseline muscle biopsies did not show any detectable skipped RT-PCR product. At Week 25, all patient biopsies showed both skipped and unskipped RT-PCR Bands. The RT-PCR products obtained were consistent with each patient’s deletion mutation and showed exon 53-specific skipping by DNA sequence analysis, supporting target engagement. The percent exon skipped as expressed in molarity and concentration is shown in Table 13 and Table 14. The 80 mg/kg/day group showed greater increases in exon skipping measured by RT-PCR compared with the 40 mg/kg/wk group.

**Table 13 Percent exon skipping by RT-PCR (expressed as Molarity)**

Percent exon skipping Molarity (nmol/L) Visit/ Statistic	40 mg/kg/wk (N=8)		80 mg/kg/wk (N=8)	
	Obs	CFB	Obs	CFB
<b>Baseline</b>				
Mean (SD)	0 (0)	--	0 (0)	--
<b>Week 25</b>				
Mean (SD)	17 (7)	17 (7)	44 (17)	44 (17)
Median	16	16	41	41
Min, Max	8,27	8,27	22,75	22,75

**Table 14 Percent exon skipping by RT-PCR (expressed as Concentration)**

Percent exon skipping Concentration (ng/μL) Visit/ Statistic	40 mg/kg/wk (N=8)		80 mg/kg/wk (N=8)	
	Obs	CFB	Obs	CFB
<b>Baseline</b>				
Mean (SD)	0 (0)	--	0 (0)	--
<b>Week 25</b>				
Mean (SD)	10 (5)	10 (5)	31 (16)	31 (16)
Median	10	10	31	31
Min, Max	4,16	4,16	12,60	12,60

The method validation was adequate to support these results, as discussed below.

#### **Method Validation for RT-PCR [OBP Reviewers-Dr. B. Aryal and Dr. A. Rao (Lead)]**

The purpose of RT-PCR method qualification was to demonstrate that (i) the established method, reagents and primers could be used for the detection of dystrophin by RT-PCR analysis, (ii) the quality RNA can be extracted from the tissue samples to convert RNA sample into complementary DNA (cDNA) and (iii) to provide qualitative evidences that the primer specific amplification of specific fragment of cDNA occurs. The RT-PCR method was qualified for the following parameters:

- Specificity
- Repeatability
- Intermediate precision

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The RT-PCR method qualification was performed by two analysts on two separate days. Both analysts used 3 non-DMD patient tissue samples for method qualification. All RNA samples passed the acceptance criteria of RNA concentration >100 ng/μL and RNA quality index (RQI) value of greater than 7. The RQI value was based on RNA quality from 1 (most degraded) to 10 (most intact) numbering system. The RQI values for all samples were more than 9.7. After confirming that the method is capable of extracting appropriate amount of quality RNA, the samples were subjected to RT-PCR. Three primer sets (44F, 46F and 48F) were designed against different exons of the dystrophin RNA transcript keeping reverse primer set constant downstream of exon 53 (54/55R) across all three primer sets as shown in the following Table 15. The positive outcome of RT-PCR measurement was confirmed by the presence of appropriate size of PCR product and consistency and repeatability of results between two analysts. The Target acceptance criteria for method qualification and results are summarized in the following Table 15. All acceptance criteria were met by both analysts.

**Table 15: Target acceptance criteria for RT-PCR method qualification**

Parameters	Target Acceptance Criteria	
<b>Repeatability</b>	<i>Pass acceptance criteria in &gt; 90% of reactions</i>	
<b>Intermediate Precision</b>	<i>Pass acceptance criteria in &gt; 90% of reactions</i>	
<b>Specificity</b>	<i>Amplicon is within ±25% of predicted size</i>	
	<b>PCR product sizes per primer set</b>	<b>bp</b>
	44F-54R product size	1603
	44F- 54/55R product size	1664
	46F- 54R product size	1258
	46F- 54/55R product size	1319
	48F- 54R product size	900
	48F- 54/55R product size	961

Source: Table 2, method qualification plan for RT-PCR exon skipping analysis for dystrophin expression page 6. (bp=base pairs)

### Analysis of Clinical samples using RT-PCR:

During clinical sample analysis, RQI and RNA concentration was confirmed to be within acceptable limits for all samples before processing for RT-PCR analysis. The RT-PCR product was loaded on the Expersion DNA 12K Analysis kit for detection. Duplicate DNA chip each containing 4 patients pre- and post-treatment samples (8 samples) were run for each set of samples. The relative skipped to un-skipped (out-of-frame) dystrophin mRNA products in the study samples was quantified using size-specific skipped and unskipped amplicon bands. The applicant states

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that the analysts performing RT-PCR were blinded to the sample information. A representative gel image provided by the applicant is given in Figure 14.

**Figure 14: Representative virtual gels from expression 12k DNA analysis chip for primer 48F and 54/55R for sample ID (b) (6) pre-infusion (left) and Wk25 (right)**



Source: Figure 28 RT-PCR, WB-IF-RT-PCR dystrophin bioanalytical report-study 201 (010-CSR-049) page 85

The applicant acknowledges that % skipped to % un-skipped determined by their method is only a relative quantitation. The direct comparison of drug-induced effect may be inaccurate and over-estimated up to 20-fold because of (i) differential effect of nonsense mediated decay (NMD) of non-functional out-of-frame mRNA transcript for the cells and (ii) differential amplification rate of skipped and un-skipped mRNA transcripts with preferential amplification of shorter skipped mRNA over longer un-skipped parental mRNA.

*OBP Reviewer's comment: Clinical samples were analyzed using a qualified RT-PCR method. I looked at the raw data and full-length gel images of clinical samples in the study reports. The skipped bands are not observed in the pre-treatment samples but faint to distinct skipped dystrophin mRNA specific bands are observed in all post-treatment samples. Nonsense-mediated mRNA decay (NMD) to prevent synthesis of abnormal proteins that can be toxic to cells and has been reported in previous literature<sup>4</sup> which may interfere with the correct interpretation of RT-PCR results. Since both NMD and the size-dependent amplification rate of mRNA transcripts were not taken into consideration for RT-PCR analysis, there may be overestimation of drug-induced effect purely based on the transcript levels reported here, therefore, RT-PCR data can only be used to confirm the mechanism of action and to support dystrophin production measured by western blot as treatment effect.*

<sup>4</sup> Khajavi et al, Nonsense-mediated mRNA decay modulates clinical outcome of genetic disease. *Eur J hum genet* 2006; 14: 1074-1081

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The overall individual dystrophin results for each method is summarized in the following Table 16 to show comparison of truncated dystrophin after treatment between methods, especially between western blot and MS.

**Table 16 Overall dystrophin results with each methodology**

Patient ID	Dose (mk/kg/wk)	Age (years)	Mutation	WB Myosin CFB	WB α-Actinin CFB	MS CFB	IF Positive Fibers CFB	% Exon skipping (molarity) CFB
(b) (6)	40	(b) (6)	50-52	8.13	3.94	2.73	8.82	12.43
(b) (6)	40	(b) (6)	47-52	10.03	10.19	1.39	11.7	26.62
(b) (6)	40	(b) (6)	49-52	4.72	8.81	2.03	12.66	10.93
(b) (6)	40	(b) (6)	49-52	4.22	4.35	2.02	4	7.54
(b) (6)	40	(b) (6)	50-52	5.06	4.58	2.55	31.5	23.41
(b) (6)	40	(b) (6)	45-52	4.19	4.19	1.42	10.66	17.56
(b) (6)	40	(b) (6)	48-52	4.39	3.87	0.63	11.9	15.02
(b) (6)	40	(b) (6)	45-52	2.76	1.67	-0.65	10.9	25.72
(b) (6)	80	(b) (6)	45-52	0.69	0.32	0.8	22.17	27.01
(b) (6)	80	(b) (6)	45-52	3.57	2.98	1.05	17.24	39.58
(b) (6)	80	(b) (6)	45-52	2.51	1.81	0.97	8.79	38.97
(b) (6)	80	(b) (6)	48-52	10.31	7.97	10.51	53.33	52.57
(b) (6)	80	(b) (6)	48-52	13.91	5.65	8.81	68.14	54.72
(b) (6)	80	(b) (6)	45-52	4.79	2.34	1.75	31.72	41.61
(b) (6)	80	(b) (6)	45-52	2.63	1.5	3.22	43.78	74.53
(b) (6)	80	(b) (6)	49-52	3.98	3.51	2.13	18.72	21.89

The highest amount of truncated dystrophin after treatment with 40 and 80 mg/kg/wk were not always consistent between methods. I also tried to look for correlation between exon deletions and the magnitude of change from baseline % dystrophin. No reliable correlation can be obtained from a small number of patients, however, patients with exon deletion 45-52 generally appeared to have lowest amounts of dystrophin produced (~3%). Patient with exon deletion of 48-52 and 50-52 generally had the highest production of dystrophin. Interestingly, this trend was also observed in Japanese Study P1/2 where patients with deletion 48-52, 50-52 and 52 had higher dystrophin production than the other patients. In the US study, there were no patients with exon 52 deletion. The numbers of patients are also too few to draw any conclusive facts, nevertheless similar trends in the two studies are noteworthy. Also, note that the Japanese study was not reviewed in this application as the analytical method for the assessment of

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dystrophin with western blot was considered inadequate, therefore any observations are mere speculations at this time (See discussion in Section 6.2).

### Functional Endpoints

In addition to the above-mentioned endpoints, time results for functional outcomes (TTSTAND, TTCLIMB, TTRW, 6MWD and NSAA) and strength assessments (QMT) were measured. TTSTAND, TTCLIMB, TTRW were reported as “time in seconds” and were converted to velocities (per second). For ease of interpreting the clinical meaningfulness of the changes observed, the Timed Functions Tests are only reported as “time in seconds” in this review (Table 17).

The following observation can be made from this open label data:

- The mean change from baseline at week 25 in the Timed Functions Tests, if any, were <0.6 seconds.
- No meaningful changes from baseline were observed in other functional tests.
- There were no meaningful dose related trends in change from baseline of functional endpoints, with the exception of mean 6MWD, which is known to be variable between assessments. A slight dose related trend was also observed for TTSTAND and NSAA, but unlikely to be clinically meaningful.

**Table 17 Change from Baseline in the Functional Endpoints**

	Treatment			
	40 mg/kg/week		80 mg/kg/wk	
	Observed	Change form Baseline	Observed	Change form Baseline
Time to Stand from Supine (TTSTAND) seconds				
Baseline				
Mean (SD)	4.17 (1.14)		4.76 (2.58)	
Week 25				
Mean (SD)	4.18 (1.65)	0.01 (1.44)	4.33 (2.67)	- 0.44
Time to 4-Stair Climb (TTCLIMB)				
Baseline				
Mean (SD)	3.90 (0.93)		3.33 (0.94)	
Week 25				
Mean (SD)	3.56 (1.58)	-0.34 (1.14)	3.33 (0.85)	0.00 (0.60)
Time to 10 m Walk/Run (TTRW)				
Baseline				
Mean (SD)	6.30 (1.58)		5.55 (1.33)	
Week 25				
Mean (SD)	5.65 (1.51)	-0.65 (1.22)	4.89 (1.05)	-0.66 (0.92)
6MWD (meters)				

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Baseline				
Mean (SD)	391.1 (33.27)		353.4 (106.32)	
Week 25				
Mean (SD)	407 (38.24)	15.6 (26.4)	407.6 (120.07)	44 (41.98)
NSAA				
Baseline				
Mean (SD)	24.8 (5.92)		23.8 (5.09)	
Week 25				
Mean (SD)	24.3 (6.16)	0.5 (3.07)	24.9 (4.52)	1.1 (2.80)

- The small changes in Functional endpoints were not correlated to the amount of dystrophin (% of normal) at Week 25. Obtaining an overall population correlation between changes and dystrophin and impact on functional tests may be difficult due to the small number of subjects; however, I explored the clinical impact of the amount of dystrophin produced in an individual patient on improvement in functional tests. Looking at individual patient data it was observed that patients with smaller % increase in dystrophin at Week 25 of <2% of normal (red cells in Table 18) were not the patients that deteriorated the most in the functional tests and patients with greater % increase in dystrophin at Week 25 of >7% of normal (Green cells in Table 18) were not the patients that showed the most improvement in the functional tests. In addition, the trends of changes observed were not the same across all timed functional tests and with NSAA and 6MWD. 6MWD has found to be variable between time point of measurement, hence I would not give much credence to the trends in 6MWD for an individual patient. Patient (b) (6) (in red) showed deterioration across many function endpoints, however this patient had dystrophin levels (~4% of normal) similar to many other patients.

Whether these magnitudes of changes in dystrophin is likely to slow progression of disease after longer duration of viltolarsen administration remains unknown and unclear. It is also not known if longer treatment results in greater amounts of dystrophin production. These are concerns that need to be addressed to understand the clinical significance of these small amounts of dystrophin produced in studies to date.

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**Table 18 Individual patient dystrophin and changes in functional tests at Week 25**

Subject	Dose	Exon Deletion	% Dystrophin (normalized to myosin)	% Dystrophin (normalized to $\alpha$ -actinin)	CFB TTRW	CFB TTCLIMB	CFB TTSTAND	CFB NSAA	CFB 6MWD
(b) (6)	40 mg	50-52	8.13	3.94	-0.1	-0.4	-1.4	6	27
	40 mg	47-52	10.03	10.19	-1.8	-1	-1.2	3	-8
	40 mg	49-52	4.72	8.81	-0.8	-0.5	-0.5	-1	56
	40 mg	49-52	4.22	4.35	1.5	2.1	2.9	-4	-7
	40 mg	50-52	5.06	4.58	-2.6	-0.4	-0.3	1	50
	40 mg	45-52	4.19	4.19	-0.9	-1.3	0.7	0	3
	40 mg	48-52	4.39	3.87	-0.4	-1.5	-0.9	1	15
	40 mg	45-52	2.76	1.67	-0.1	0.3	1.1	-2	-11
(b) (6)	80 mg	45-52	0.69	0.32	-0.1	-0.1	0.8	-1	73
	80 mg	45-52	3.57	2.98	-0.3	-0.3	-0.5	2	24
	80 mg	45-52	2.51	1.81	-0.7	0.4	0.1	-3	85
	80 mg	48-52	10.31	7.97	0.2	0.1	0.1	-1	-1
	80 mg	48-52	13.91	5.65	-2.8	-1	-1.2	3	-
	80 mg	45-52	4.79	2.34	-0.8	-0.2	-1	4	41
	80 mg	45-52	2.63	1.5	-0.5	1.1	-0.4	0	-10
	80 mg	49-52	3.98	3.51	-0.3	0	-1.4	5	96

CFB=change from baseline

Green cells indicate higher amounts of dystrophin of >7% and improvement of >1 second in Timed Function Test, >3 points on NSAA and >50 m on 6MWD

Red cells indicate lower amounts of dystrophin of <2% and deterioration of >1 second in Timed Function Test and > 3 points in NSAA

**Comparison of functional endpoints to natural history:**

The applicant also compared these functional endpoints to CINRG network natural history patients (9 exon 53 skipping patients, 56 non-exon 53 skipping patients i.e. total of 65 Natural history patients). applicant states that Study 201 was conducted at clinical sites participating in the CINRG network, so the SOPs (clinical manuals) and clinical evaluator (CE) training protocols were harmonized between Study 201 and the CINRG natural history database, however there are many known as well as unknown factors that cannot be accounted for in such comparisons

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given the heterogeneity of the disease and patient and care characteristics. Given the imprecision of population matching due to lack of control of all known and unknown biases, a rationale for establishing clinical benefit with such comparison does not appear to be justified. The applicant's analysis did not show any clinically meaningful difference in clinical function at the end of 24 weeks of treatment with viltolarsen 40 and 80 mg/kg/wk compared to natural history or any consistent dose related trends in any functional endpoints.

### **Dose/Dose Response**

No dose-response was observed in dystrophin expression by western blot, with change from baseline dystrophin being greater in the 40 mg/kg/wk compared to the 80 mg/kg/wk dose. These differences were not statistically significant. Dystrophin with MS methodology non-significant trend of being greater in the 80 mg/kg/wk dose group. The mean and median dystrophin positive fibers were higher in the 80 mg/kg/wk group, there were no statistical difference between the doses.

### **Durability of Response**

Not known

### **Persistence of Effect**

Not known

## **6.2 Study NS065/NCNP01-P1/2 (referred as Study P1/2 in the review)**

The applicant submitted another multicenter, parallel-group, comparative, open-label 24-week study in 16 patients (ages  $\geq 5$  and  $<18$  years) with two dose groups: 40 or 80 mg/kg once weekly that was conducted in Japan as a supportive study, with dystrophin assessments by western blot, immunofluorescence and exon skipping efficiency by RT-PCR as primary endpoints. Muscle biopsies of the left or right tibialis anterior muscle or biceps brachii muscle was taken at baseline and Week 12 from 8 subjects and at Week 24 from 8 subjects at both dose groups.

This study was not reviewed for dystrophin assessments as the method validation for each of the methodologies was considered inadequate and not comparable to the bioassay used in Study 201 (based on OBP review by Drs. Aryal and Rao) as summarized below.

### **Inadequacy of method validation [OBP Reviewers-Dr. B. Aryal and Dr. A. Rao (Lead)]**

*OBP Reviewer's Comment:*

*The lack of robustness of the methods is based on these different attributes:*



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*Therefore, overall, the quality of the dystrophin data from Japanese Phase 1/2 study are considered not robust and inconclusive to make any claims* (b) (4)

### 6.3. Study NS-065/NCNP-01-202

An ongoing 144-week open label extension of Study 201 was submitted to support long-term safety of viltolarsen in 16 subjects. No efficacy data from this study was submitted in the application.

## 7 Integrated Review of Effectiveness

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### 7.2 Assessment of Efficacy Across Trials

A full integrated review and assessment of efficacy was not warranted as the efficacy based on

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dystrophin as a surrogate endpoint was established from a single US/Canada Study 201 in 16 subjects after 24 weeks of dosing with 40 and 80 mg/kg/week viltolarsen. The criteria for accelerated approval of viltolarsen is discussed in section 7.4.

The population of this study is small and relatively homogenous; hence subgroup assessment of efficacy is not warranted.

### **7.3 Additional Efficacy Considerations**

#### **7.3.1 Considerations on Benefit in the Postmarket Setting**

A confirmatory study is currently recruiting patients to confirm clinical benefit of viltolarsen 80 mg/kg/wk with Time To Stand as the primary functional endpoint.

### **7.4 Integrated Assessment of Effectiveness**

The applicant is seeking accelerated approval of viltolarsen for the treatment of DMD in patients who have confirmed mutation of the DMD gene that is amenable to exon 53 skipping based on dystrophin a surrogate marker. The application included a 24-week Study 201 with dystrophin assessments with various methodologies as the primary endpoint and secondary endpoints. The application also included a similar 24-week study conducted in Japan with dystrophin quantification as supportive data, however, given the inadequacy of assay methodologies, this study was not reviewed. Supportive evidence of efficacy can be derived from the secondary dystrophin assessment using mass spectrometry methodology from Study 201.

In Study 201, muscle biopsies were taken from biceps brachii muscle at baseline and Week 25 in 16 DMD patients (8 in each dose group, 40 and 80 mg/kg/week). The primary endpoint was percent of normal dystrophin analyzed by western blot. The mean baseline dystrophin protein measured by western blot was 0.3% (range 0.1-0.4) and 0.6% (range 0.1-2.6) of normal for viltolarsen 40 and 80 mg/kg/week, respectively when normalized to myosin heavy chain.

After 24 weeks of treatment, the mean change from baseline dystrophin was 5.4% (range 2.8-10%) and 5.3% (0.7-13.9%) of normal for viltolarsen 40 and 80 mg/kg/week, respectively when normalized to myosin heavy chain. There was no dose related difference in dystrophin production at the 40 and 80 mg/kg/week dose. The median change in dystrophin was 4.6 and 3.8% of normal for viltolarsen 40 and 80 mg/kg/week, respectively when normalized to myosin heavy chain.

The median change in dystrophin was 1.7 and 1.9% of normal for viltolarsen 40 and 80 mg/kg/week, respectively when analyzed using a validated mass spectrometry method (secondary endpoint).

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The accelerated approval provisions of FDASIA in section 506(c) of the FD&C Act provide that FDA may grant accelerated approval to:

... a product for a serious or life-threatening disease or condition ... upon a determination that the product has an effect on a surrogate endpoint that is reasonably likely to predict clinical benefit, or on a clinical endpoint that can be measured earlier than irreversible morbidity or mortality, that is reasonably likely to predict an effect on irreversible morbidity or mortality or other clinical benefit, taking into account the severity, rarity, or prevalence of the condition and the availability or lack of alternative treatments.

The qualifying criteria for accelerated approval include:

1. Serious condition: Viltolarsen application meets this criterion as DMD is a life-threatening disease.
2. Meaningful advantage over available therapy: The accelerated approval regulations state that accelerated approval is available only for drugs that provide a meaningful therapeutic benefit over existing treatments. Currently, there are 3 approved treatments for DMD:
  - a. Exondys<sup>®</sup>51 (Eteplirsen): Eteplirsen is indicated for patients who had a confirmed mutation in the DMD gene that is amenable to exon 51 skipping. This is a different population than that proposed for viltolarsen (for patients amenable to exon 53 skipping), hence does not count as available therapy.
  - b. Emflaza<sup>®</sup> (Deflazacort): Emflaza is a steroid that is indicated for the treatment of DMD irrespective to the mutation in DMD gene. It is used as a standard of care for most DMD patients, therefore viltolarsen was administered to patients that were all on steroids, either deflazacort or prednisone. In Study 201, 12/15 patients were on deflazacort and the remaining 4 on prednisone. The regulations provide criteria where an alternative therapy with efficacy comparable to available therapy, but with a different mechanism of action, could be of added clinical value in a disease setting in which a significant number of patients may respond differently to the new therapy. The criteria defining unmet need allows for mechanistic diversity, even without a documented efficacy or safety advantage that could be advantageous in disease settings in which drugs become less effective or ineffective over time. In spite of steroid use, the patients progress in their disease over time. Although viltolarsen meets the flexibility criteria of a distinct mechanism from that of deflazacort, whether it is likely to provide added efficacy advantage remains unclear, however, it could have potential for added benefit (see discussion of criteria 3 for accelerated approval)
  - c. Vyondys<sup>®</sup>53 (Golodirsen): Vyondys<sup>®</sup> is indicated for patients who had a confirmed mutation in the DMD gene that is amenable to exon 53 skipping. This indication is identical to that for viltolarsen. However, a new therapy can be considered advantageous over available therapy, where the available therapy was approved under accelerated approval program based in a surrogate endpoint where the clinical benefit as not been verified. Golodirsen received accelerated approval, there does

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not count as available therapy until clinical benefit is established in a confirmatory study.

Therefore, viltolarsen does meet the criteria of added benefit over existing therapy.

### 3. Demonstrates an effect on a surrogate or an intermediate clinical endpoint that is reasonably likely to predict clinical benefit:

As indicated in the Guidance for Industry for Expedited Programs for serious condition-Drugs and Biologics, *determining whether an endpoint is reasonably likely to predict clinical benefit is a matter of judgment that will depend on the biological plausibility of the relationship between the disease, the endpoint, and the desired effect and the empirical evidence to support that relationship. Accelerated approval provisions allows empirical evidence to include “. . . epidemiological, pathophysiological, therapeutic, pharmacologic, or other evidence developed using biomarkers, for example, or other scientific methods or tools.” Evidence of pharmacologic activity alone is not sufficient, however (57 FR 58942). Clinical data should be provided to support a conclusion that a relationship of an effect on the surrogate endpoint or intermediate clinical endpoint to an effect on the clinical outcome is reasonably likely.*

At the Type C meeting in May 2018, the Agency noted that the applicant must provide in the NDA evidence that truncated dystrophin produced by viltolarsen were at levels reasonably likely to predict clinical benefit. The applicant addresses this by providing epidemiological and pathophysiological evidence from scientific literature that a milder DMD or BMD phenotype results from dystrophin levels similar to that produced by viltolarsen. I will further discuss, the applicant's view point in this section.

Before I discuss, the applicant's rationale for asserting the likelihood that dystrophin produced by viltolarsen will predict clinical benefit, I would like to acknowledge that the Agency has established prior precedent of accelerated approval of antisense oligonucleotides based on an increase in dystrophin protein as a surrogate endpoint that is reasonably likely to predict benefit in DMD patients. The two prior approvals based on this are: (1) EXONDYS 51® (eteplirsen, NDA 206488) (2) VYONDYS 53® (golodirsen, N211970).

To support the reasonable likelihood of clinical benefit, the applicant cites the following evidences:

- **Evidence from BMD patients:** The applicant presents its argument that dystrophin levels  $\geq 3\%$  as seen in BMD patients will mitigate symptoms of DMD providing clinically meaningful improvements in function. To support this the applicant cites the first extensive study of correlations of dystrophin content of muscle and clinical phenotype in 97 patients with possible Becker muscular dystrophy<sup>5</sup>, where the applicant presents the following conclusion

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<sup>5</sup> Hoffman et al. (1989) Improved diagnosis of Decker muscular dystrophy by dystrophin testing, Neurology 39:1011-1017

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from the authors: “The correlation of both the biochemical and clinical data suggests that Duchenne/Becker dystrophy can be divided into 4 clinically useful categories: Duchenne dystrophy (wheelchair at about age 11 years; dystrophin quantity less than 3% of normal); severe Becker dystrophy (wheelchair age 13 to 20 years; dystrophin 3% to 10%); and moderate/mild Becker dystrophy (wheelchair greater than 20 years; dystrophin quantity greater than or equal to 20%).”

I (VT) now present my argument why this published evidence does not support the reasonable likelihood of clinical benefit with viltolarsen. While broadly it is logical to believe that higher amounts of dystrophin will likely lead to milder phenotype as observed in BMD patients who present higher levels of dystrophin compared to DMD patients, however, the minimum threshold of dystrophin that will alter the clinical phenotype of DMD patients remains unclear at this time. Even after keeping in mind the differences in dystrophin quantification methodologies, beyond broad distinctions, any correlation between dystrophin levels and functionality is limited from this publication due to the following reasons:

- The published article cited by the applicant only included possible BMD patients, so reference to DMD patients in the above paragraph is inferred from other published data. The referenced article does not include patients with <10% of dystrophin (only 1 patient with 5% dystrophin was included).
- The published article suggests that patients with the same amount of dystrophin (i.e. 10% of normal) can become wheelchair bound at ages between 15 and 23 indicating phenotypic variability in time to loss of ambulation (LOA) with the same amount of dystrophin.
- The article also showed that patients can lose ambulation by age 15-16 years with either 5 or 10% of normal dystrophin, although I acknowledge that there was only one BMD patient with 5% dystrophin in the referenced study. In addition, patients with dystrophin levels of 20%, 90% or 100% of normal dystrophin presented with similar severe clinical presentation, again suggesting that the clinical presentation did not depend entirely on the amount of dystrophin in BMD patients.

Researchers have shown that variable nature of BMD mutations that affect the central rod domain or the N-terminal actin binding domain of the dystrophin gene have made it difficult to show a correlation between levels of dystrophin in BMD and disease severity. In addition to dystrophin it is known that steroid use over 1 year can prolong the time to lose ambulation to >15 years in many DMD patients that have much lower dystrophin levels. For example, for exon 53 skippable patients that are on steroids, the median age (95% CI) at LOA is 14 (9-17.2 years) with much lower baseline dystrophin<sup>6</sup>. Note that the referenced study in BMD patients is prior to the era of steroid treatment.

It has been suggested by many researchers that it is not only the amount of dystrophin, but

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<sup>6</sup> Bello et. al.; DMD genotypes and loss of ambulation in the CINRG Duchenne Natural History Study. *Neurology*. (2016) Jul 26; 87(4):401-9.

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other factors such as differential stability of the truncated protein, uniformity of dystrophin expression, muscle content of the biopsy sample, muscle inflammation, micro environment surrounding individual muscle fibers, cycles of fiber necrosis and regeneration, fiber loss, genetic modifiers leading to milder or more severe phenotypes with similar levels of proteins, or endogenous exon skipping, to name a few factors that complicate the understanding regarding levels of dystrophin that will be clinically meaningful. These complications also suggest that increase in expression of dystrophin alone may not be predictive of clinical benefit in patients. Demonstration of a shift in key disease milestones in large number of patients may allow the establishment of a threshold of increases in dystrophin levels that are likely to predict clinical benefit. Understandably, this can be very challenging.

There has been no clear consensus on the minimum levels of dystrophin that are required for appropriate muscle integrity and function that will change how a patient feels, functions and survives. Earlier studies have shown that dystrophin as low as 30% are sufficient to prevent development of muscular dystrophy, however it should be noted that methodologies of dystrophin assessment in some published studies are not comparable to Agency's current standards<sup>7</sup>. Based on mouse models of DMD, it has been hypothesized that dystrophin levels of >20% of normal levels with a uniform expression are likely needed to provide clinical benefit to patients<sup>8</sup> or even a minimal amount of 10% of normal would likely be needed for clinical benefit<sup>9</sup>. Case reports of patients are published where dystrophin as low as 3.2% of normal had a mild phenotype, however this patient was homozygous with *LTBP4* haplotype that is associated with prolonged ambulation and a milder phenotype<sup>10</sup>. All these appear to suggest larger amounts of dystrophin than that observed with viltolarsen will likely predict clinical benefit in DMD in the absence of any genetic modifiers of the milder phenotype and probably remains a matter of judgement.

In conclusion, the published article cited by the applicant does not provide conclusive evidence that the levels of dystrophin produced by viltolarsen are likely to predict clinical benefit in patients and whether dystrophin alone can explain clinical severity in a patient and the likelihood of clinical benefit.

- **Evidence from DMD patients of milder phenotype:** Secondly, the applicant provides evidence from DMD patients with a milder phenotype to justify the reasonable likelihood of

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<sup>7</sup> Neri et., al, Dystrophin levels as low as 30% are sufficient to avoid muscular dystrophy in the human. *Neuromuscul Disord* (2007) 17(11–12):913–918

<sup>8</sup> Wells, What levels of dystrophin expression are required for effective therapy of DMD; (2019); *Journal of Muscle Research and Cell Motility* (2019) 40:141–150

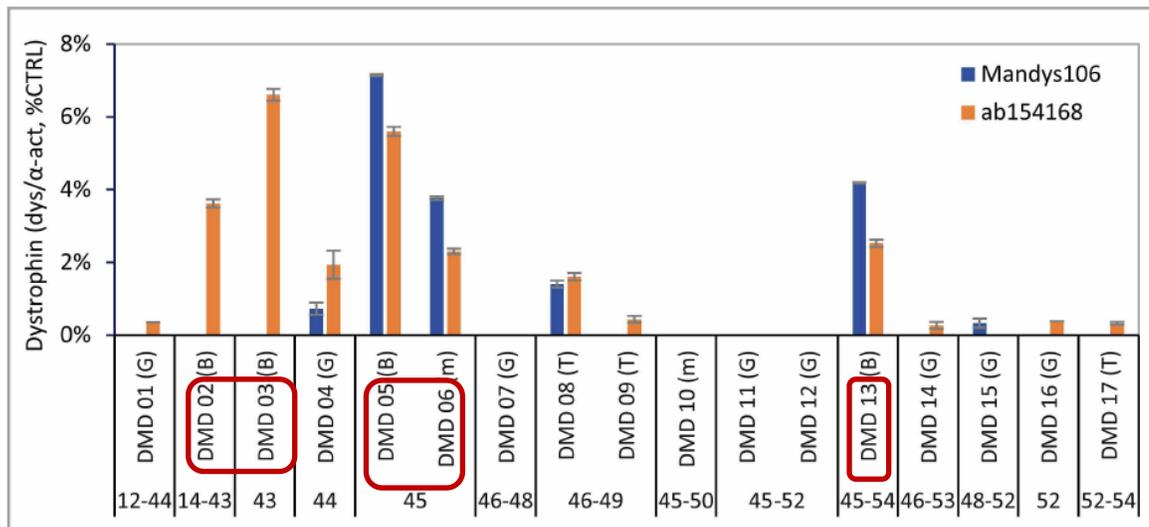
<sup>9</sup> JC van den Bergen JC, et al. Dystrophin levels and clinical severity in Becker muscular dystrophy patients; *J Neurol Neurosurg Psychiatry* 2013;0:1–7. doi:10.1136/jnnp-2013-306350

<sup>10</sup> Waldrop e. al ; Low-level dystrophin expression attenuating the dystrophinopathy Phenotype; 2018) *Neuromuscular Disorders* 28 (2018) 116–121

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dystrophin levels produced by viltolarsen to predict clinical benefit the DMD patients amenable to exon 53 skipping. To support this, the applicant cites a recent publication where muscle dystrophin levels with the sensitive and reliable capillary western immunoassay was shown in 17 DMD patients. Applicant justifies that the same anti-dystrophin antibodies and  $\alpha$ -actinin controls that were utilized for viltolarsen clinical development were also utilized in this study. The study reported baseline dystrophin levels of 3-7% in a few DMD patients (Patient numbers 02, 03, 05, 06 and 13 in the Figure below) that were exon 44 flanking deletion DMD patients in the Figure below from the publication<sup>11</sup>.



**Fig 7. Dystrophin levels in skeletal muscle samples derived from DMD patients.** Panel of 17 gastrocnemius, biceps and tibialis muscle samples from DMD patients, analysed for dystrophin using ab154168 and Mandys106 and normalized to  $\alpha$ -actinin (n = 2). Protein loading was 1.25  $\mu$ g. Muscle types are indicated between brackets: G = gastrocnemius; B = biceps; T = tibialis and M = miscellaneous.

<https://doi.org/10.1371/journal.pone.0195850.g007>

Source: Beekman et. al 2018

The applicant's argument is that since Exon 44 flanking deletion DMD patients have also been shown to have a longer time to loss of ambulation compared to some other exon skippable mutations in a few publications<sup>12,5</sup>; the higher amounts of dystrophin (close to the levels seen in the above study) as that produced by viltolarsen will provide meaningful improvements in function.

I (VT) will now present my argument on applicant's rationale based on this evidence from the scientific literature. It appears a logical inference to draw from the example of Exon 44 patients

<sup>11</sup> Beekman et.al; Use of capillary Western immunoassay (Wes) for quantification of dystrophin levels in skeletal muscle of healthy controls and individuals with Becker and Duchenne muscular dystrophy; April 11, 2018

<https://doi.org/10.1371/journal.pone.0195850> April 11, 2018

<sup>12</sup> Van den Berger et. al. Prolonged Ambulation in Duchenne Patients with a Mutation Amenable to Exon 44 Skipping. J Neuromuscul Dis. 2014;1(1):91-94.

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that are known to be of milder phenotype in various publications; however, no direct correlation between these higher amounts of dystrophin and improved clinical function in these patients can be obtained from the cited publication as clinical function was not assessed in these patients. It has been suggested that patients with these types of mutations present with unpredictable phenotypes.<sup>13</sup> Published studies have shown variable age of loss of ambulation in exon 44 flanking patients. For example, the age at wheelchair dependence was reported as 10.8 years in exon 44 flanking deletion DMD patients (N=33) compared to 9.8 years for others, N=81<sup>11</sup> by some authors (van Den Berger 2014), yet others (Bello 2016) have shown this to be a median of 14.8 years in these patients (N=20)<sup>5</sup>. Anthony et. al<sup>12</sup> have also shown that out of frame exon 44 flanking deletions result in a variety of clinical severity with variable age of loss of ambulation (11-17 years as shown in the Table below from the publication). Wang et. al. suggest that Exon 44 flanking patients, particularly with single mutation type consisting of exon 45 deletion showed a median loss of ambulation of 20 years (N=49) compared to other exon 44 flanking patients and therefore suggested that milder phenotype in the exon 44 skippable patients may be restricted to the exon 45 deletion subgroup in exon 44 flanking patients (Wang 2018).<sup>14</sup> A direct correlation between the amount of dystrophin to the age of loss of ambulation is not known from these patients in these publications. However, looking at the data published by Anthony et. al. there appears to be phenotypic variability within exon 45 deletion subgroup as well, showing the age of loss of ambulation ranging from 11-17 years (shown in the subsequent Table from the publication). Given the variability in age of loss of ambulation in the various publication and the lack of a direct correlation between the amount of dystrophin to the age of loss of ambulation, it is difficult to conclude that higher amounts of dystrophin alone will relate to higher clinical benefit in this subgroup of exon 44 flanking patients.

Patient No.	Exon Deletion	Exon Skipping Model	Frame	Age at Biopsy, y	Age at Onset	Phenotype	Symptoms	Motor Function
1	42-43	44	OOF	7	6 y	IMD	Unable to jump	Walking indoors at age 15 y
2	42-43	44	OOF	9	3 y	IMD	Motor delay	Walking indoors at age 14 y
3	45	44	OOF	8	Unknown	IMD	Toe walking	Ambulant at age 17 y, not running, difficulty climbing stairs
4	45	44	OOF	3	2 y	DMD	Frequent falls	LOA at age 11 y
5	45	44	OOF	1	17 mo	DMD	Delayed walking, diagnosed by incidental high serum CK level	LOA at age 12 y

Source: Anthony et al (2013)

Some researchers have given a biological explanation that the more mildly affected patients such as exon 44 flanking deletion DMD patients may have a higher frequency of revertant fibers

<sup>13</sup> Anthony et.al. Biochemical characterization of patients with In-frame or Out-of-frame DMD deletions pertinent to exon 44 or 45 skipping; *JAMA Neurol.* doi:10.1001/jamaneurol.2013.4908 Published online November 11, 2013

<sup>14</sup> Wang et. al. *DMD Genotype Correlations from DuchenneConnect: endogenous exon skipping is a factor in prolonged ambulation for individuals with a defined mutation sub-type*, 2018 (Accepted article)

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and trace dystrophin due to more frequent spontaneous skipping of exon 44 that generates in frame transcripts and resulting functional dystrophin.<sup>15, 16, 17, 18</sup> The higher percentage of dystrophin in these patients include the larger number of revertant fibers that cannot be differentiated by the assay methodologies used in the publications.

We should also bear in mind that the difference in the western blot method used in this publication compared to that in study 201, therefore such cross-assay comparison of dystrophin levels may not be completely reliable either. It is also not known if the muscle integrity and motility are the same with spontaneous mutations resulting in revertant fibers versus the drug-induced truncated dystrophin.

Additionally, in BMD patients, dystrophin levels have been shown to vary from 3% to 100% of a healthy control, with variable clinical phenotypes which don't always correlate.<sup>19</sup> Therefore, to assert any such correlation with milder DMD mutations would require larger number of subjects where levels of dystrophin and time to loss of ambulation (or other key milestones that affect daily functioning) are known. Even then, methods within western blot may differ with studies, and therefore, standardization of the assay methods may further the science in this area.

Also noteworthy, is that all these patients that showed higher amounts of dystrophin had biopsies taken from biceps where as other patients had biopsies from tibialis anterior or gastrocnemius muscles. Animal studies have shown that de novo dystrophin varied highly between PMP-treated mdx animals and between type of muscles ranging from 0-80% of wild type controls, where the triceps muscle showed the highest degree of rescue with an antisense oligonucleotide, lower amounts in tibialis anterior and heart muscles being the lowest. Variable levels are also observed between individual myofibers in the same muscle sample.<sup>20</sup> The sample size is not large in the published data on baseline dystrophin levels in different skeletal muscle types to draw firm conclusions of a trend of higher baseline dystrophin being related to exon 44 flanking deletion patients or the biceps muscle of DMD patients, but coincidentally it is noteworthy that these exon 44 flanking patients that showed higher amounts of dystrophin had biopsies on the biceps muscle.

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<sup>15</sup> Anthony et.al. Biochemical characterization of patients with In-frame or Out-of-frame DMD deletions pertinent to exon 44 or 45 skipping; *JAMA Neurol*. doi:10.1001/jamaneurol.2013.4908 Published online November 11, 2013

<sup>16</sup> Wang et. al. *DMD Genotype Correlations from DuchenneConnect: endogenous exon skipping is a factor in prolonged ambulation for individuals with a defined mutation sub-type*, 2018 (Accepted article)

<sup>17</sup> Bello et. al (2016); *DMD genotypes and loss of ambulation in the CINRG Duchenne Natural History Study*. *Neurology*. 2016 Jul 26;87(4):401-9.

<sup>18</sup> Van den Berger et. al. *Prolonged Ambulation in Duchenne Patients with a Mutation Amenable to Exon 44 Skipping*. *J Neuromuscul Dis*. 2014;1(1):91-94.

<sup>19</sup> JC van den Bergen JC, et al. *Dystrophin levels and clinical severity in Becker muscular dystrophy patients*; *J Neurol Neurosurg Psychiatry* 2013;0:1–7. doi:10.1136/jnnp-2013-306350

<sup>20</sup> Vila MC et al *Elusive sources of variability of dystrophin rescue by exon skipping*. *Skelet Muscle* (2015) 5:44. <https://doi.org/10.1186/s13395-015-0070-6>

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In conclusion, there is no direct correlation between amount of dystrophin and clinical function in the exon 44 flanking patients. The time of loss of ambulation in the milder DMD phenotype comprising of exon 44 flanking patients is variable ranging from 10.8-20 years in published studies, so no clear inference can be drawn that the milder phenotype is related to the amount of dystrophin in these patients. In addition, there is a confounding factor of the muscle type of the biopsy (biceps) that could possibly show higher amounts of dystrophin in the exon 44 flanking patients. Therefore, I see no conclusive interpretation that the amount of dystrophin produced by viltolarsen is likely to predict clinical benefit based on the published literature presented by the applicant.

- **Evidence from viltolarsen clinical data compared to natural history:** As yet another argument to support the reasonable likelihood to predict clinical benefit with viltolarsen, the applicant has compared change from baseline in Time Function Tests, 6MWD and NSAA compared to CINRG Natural history subjects. The applicant's argument lends no credence in establishing clinical benefit with such comparison, given the imprecision of population matching due to lack of control of all known and unknown biases and selection bias of the retrospectively collected natural history control population.

However, I tried to look for correlation or trends in increase in dystrophin and the changes in functional tests from baseline to Week 25 in the study for individual patients. The magnitude of an increase in dystrophin protein was not correlated to a proportional magnitude of improvement in functional tests for individual patients based on change from baseline at Week 25. Patients with a change from baseline of >7% of normal dystrophin after 24 weeks of treatment were not the patients that improved the most in the functional assessments. Similarly, the patients with lower amounts of dystrophin of <2% were not the patients that deteriorated the most (Table 18 in the Individual Study review). A population correlation between dystrophin and improvement in function may require larger number of patients, however, any increase in protein should impact the clinical function in an individual patient. Overall the improvement in timed function tests was less than 1 second in majority of the subjects (mean improvement of <0.6 seconds across all time function tests). Therefore, the data do not support that the amount of dystrophin produced after 24 weeks of treatment is reasonably likely to predict clinically meaningful benefit. However, it is not known if treatment for longer duration will have a greater impact on improvement in clinical function and likely prolong ambulation in these patients and if longer duration treatment can increase in the amount of dystrophin in muscle fibers.

The applicant has also included evidence from Female Carriers of DMD and Animal DMD models, which appear remote in being able to predict clinical benefit in DMD patients, hence I do not discuss them here.

I have the following additional concerns regarding increase in dystrophin as produced by viltolarsen being likely to predict clinical benefit based on data submitted in the application:

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- Pre-clinical data have shown that there is a strong dose effect of ASOs, with high levels of oligonucleotide drug leading to greater de novo dystrophin production overall<sup>21</sup>. A statistically significant dose effect on dystrophin production was not observed in Study 201 with western blot analysis or any other analyses. It is unclear if this is due to saturation of effect at 40 mg/kg/wk.
- There is no clear presentation of baseline dystrophin expression in DMD patients, but reported to be <3%, 0-5% 0.4-7%<sup>22</sup> in various publications (note: assay methodologies vary). There are no published studies defining the clinical presentation of patients with baseline dystrophin values >2% of normal compared to patients with 0.05% of normal at baseline to understand if small increases in dystrophin post treatment with drugs to induce dystrophin production are likely to be clinically meaningful. It is promising to note that, with 24 weeks of treatment with viltolarsen, 88% of the patients had a post-treatment truncated dystrophin >3% of normal and 44% had post-treatment truncated dystrophin expression >5% of normal (if we consider baseline dystrophin in DMD patients to be either <3% or <5% of normal). This indicates that viltolarsen increased dystrophin levels beyond that reported as baseline levels in DMD patients; however, an impact on clinical function was not visible within the 24 weeks study duration. It is not clear if this suggests that, yet higher amounts of dystrophin would impact clinical function or there are other factors in the muscle pathology that are yet to be identified or a longer duration of treatment would be needed to demonstrate a change in phenotype.

In summary, I do not find clear evidence in this application that the amount of dystrophin produced by viltolarsen is reasonably likely to predict clinical benefit. Nevertheless, there are two prior approvals granted for the treatment of DMD in patients amenable to exon 51 and exon 53 skipping that serve as precedents of specifically concluding that small amounts of truncated dystrophin was reasonable likely to predict clinical benefit: (1) EXONDYS 51<sup>®</sup> (NDA 206488) that showed a mean ( $\pm$ SD) increase in dystrophin from 0.16%  $\pm$  0.12% of normal to 0.44%  $\pm$  0.43% ( $p < 0.05$ ) of normal and median increase of 0.1% after 48 weeks of treatment ( $n=12$ ); (2) VYONDYS 53<sup>®</sup> (N211970) that showed a mean ( $\pm$ SD) increase in dystrophin from 0.1%  $\pm$  0.07% of normal to 1.02%  $\pm$  1.03% ( $p < 0.001$ ) of normal and median increase of 0.88% after 48 weeks of treatment ( $N=25$ ) (data from Product labels).

Viltolarsen at 40 mg/kg/day has shown a mean ( $\pm$ SD) increase in dystrophin from 0.3%  $\pm$  0.1% of normal to 5.7%  $\pm$  2.4% ( $p = 0.0004$ ) of normal and median increase of 4.6% after 24 weeks of treatment ( $n=8$ ). Viltolarsen at 80 mg/kg/day has shown a mean ( $\pm$ SD) increase in dystrophin

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<sup>21</sup> Alter J, Lou F, Rabinowitz A, Yin H, Rosenfeld J, Wilton SD, et al. Systemic delivery of morpholino oligonucleotide restores dystrophin expression body wide and improves dystrophic pathology. *Nat Med*. 2006;12(2):175–7.

<sup>22</sup> Beekman et.al; Use of capillary Western immunoassay (Wes) for quantification of dystrophin levels in skeletal muscle of healthy controls and individuals with Becker and Duchenne muscular dystrophy; <https://doi.org/10.1371/journal.pone.0195850> April 11, 2018

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from  $0.6\% \pm 0.8\%$  of normal to  $5.9\% \pm 4.5\%$  ( $p = 0.01$ ) of normal and median increase of 3.8% after 24 weeks of treatment ( $n=8$ ) when normalized with myosin. The mean (SD) change from baseline dystrophin were similar at the two doses, with an increase of  $5.4\% \pm 2.4\%$  and  $5.3\% \pm 4.5\%$  of normal with the 40 and 80 mg/kg/wk doses, respectively. Based on the dystrophin results and the prior precedents, I recommend granting accelerated approval of viltolarsen as well that has shown the production of truncated dystrophin after 24 weeks of treatment. The application supports a single study approval based on statistically persuasive results on the primary endpoint across doses with additional support from secondary endpoints.

The applicant is seeking approval of the 80 mg/kg/week dose of viltolarsen. Although truncated dystrophin based on the western blot analyses suggested no dose difference, a secondary endpoint for dystrophin quantification mass spectrometry also showed an increase in dystrophin at the two doses that was not statistically different, with a mean $\pm$ SD change from baseline dystrophin of  $1.5\% \pm 1.1\%$  and  $3.7\% \pm 3.8\%$  of normal with the 40 and 80 mg/kg/wk doses, respectively and a median increase of 1.7% and 1.9% of normal for the 40 and 80 mg/kg/wk doses respectively. There was also no notable difference in safety between the two doses (see Section 8). Given, the marginal better results with the 80 mg/kg/week dose, it appears reasonable to approve the 80 mg/kg/wk dose.

A 48-week placebo-controlled confirmatory trial (NS-065/NCNP-01-301) with 80 mg/kg/wk dose is initiated and will be able to provide evidence of clinical benefit in the future with Time to Stand as the primary function endpoint.

## 8 Review of Safety

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### 8.2 Safety Review Approach

The safety population consisted of all randomized patients who received at least 1 dose of viltolarsen.

There are 3 clinical studies that contribute to the safety information for viltolarsen injection:

- Study 201 conducted in US/Canada providing primary safety data at viltolarsen doses of 40 and 80 mg/kg/wk. This study included an initial 4-week placebo-controlled part followed by open label-dosing for additional 20 weeks. Therefore, the placebo-controlled safety data in the development program of viltolarsen is limited to only 4 weeks. Overall, this study provided 20-24 weeks of safety data.
- Study 202 providing long-term safety data for a total of 73-107 weeks of exposure based on a cut-off date of 29 January 2019 of an ongoing 144-week extension study of Study 201.
- Study P1/2 conducted in Japan providing supportive open label safety data for 24 weeks at viltolarsen doses of 40 and 80 mg/kg/wk.

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In addition to these, safety findings from a 12-week Phase 1 study DMT101 in 10 patients that evaluated doses 1.25-20 mg/kg/wk has been discussed in the review where relevant. The individual studies are tabulated and described in Sections 5.1, 6.1 and 6.2 in the review of clinical efficacy.

The overall approach to safety review will be focused mostly on the open label studies. The controlled data is limited to very small number of subjects for a treatment duration of only 4 weeks. However, this limited controlled safety data will also be discussed in the review. The limitation of this approach is that without quantitative comparisons of risk to placebo, it will be difficult to assess whether any adverse event (AE), vital sign change or laboratory result was due to drug, or merely a background event that would have been observed in the absence of treatment.

Overall safety will be assessed in the following groups:

1. Analysis of controlled safety database: duration of 4-weeks
2. Pooled analysis of Study 201 and P1/2: duration of 20-24 weeks
3. Long term safety analysis of Study 202: duration of 70-107 weeks based on a cut-off date of January 29, 2019 from the 144-week ongoing study

### 90-Day Safety Update (agreed by FDA in communication dated August 27, 2019):

The 90-Day safety update included a cut-off of July 5, 2019 that provided additional 22 weeks of safety data representing 95-129 weeks of exposure of the US/Canada patients.

### **Review Strategy for Safety Issues of Special Interest:**

The drug-specific events of interest discussed in the review include injection site reactions and effects on renal function.

## **8.3 Review of the Safety Database**

### **8.3.1 Overall Exposure**

Given that viltolarsen is a rare disease, the exposure in the development program is understandably far less than that recommended by the ICH guidelines. However, there is no specific minimum number of patients that should be studied to establish clinical safety. Given the rarity of DMD disease and that the applicant is seeking accelerated approval based on a surrogate endpoint, this limited safety data was deemed acceptable at the Pre-NDA meeting.

A total of 32 DMD patients were exposed to viltolarsen 40 and 80 mg/kg/wk for 20-24 weeks. A total of 16 of these 32 patients were exposed to viltolarsen for a duration >1 year in an ongoing study (Study 202). The overall exposure is summarized in Table 19.

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**Table 19 Overall exposure of viltolarsen**

Safety Database for the Study Drug <sup>1</sup>			
Individuals exposed to any treatment in this development program for the indication under review			
N=32			
(N is the sum of all available numbers from the columns below)			
Clinical Trial Groups	Viltolarsen 40 mg/kg/wk	Viltolarsen 80 mg/kg/wk	Placebo
Healthy volunteers <sup>1</sup>	0	0	0
Controlled trials conducted for this indication <sup>2</sup>	6	5	5**
Uncontrolled trials <sup>3</sup> conducted for this indication <sup>3</sup>	10	11	NA

<sup>1</sup>A total of 10 Healthy volunteer study DM101 tested doses up to 20mg/kg/wk for 12 weeks

<sup>2</sup>The controlled part of the study was for 4 weeks.

<sup>3</sup>The uncontrolled trials included both US Study 201 and Japanese Study P1/2

\*\*Two placebo subjects switched to 40 mg/kg/wk after 4 weeks and three switched to 80 mg/kg/wk. These placebo subjects contributed to 20 weeks of safety data.

In the pooled Phase 2 studies, 32 patients were exposed to viltolarsen for a mean of 12 months (range: 5.3-24.5 months). Sixteen patients were treated for at least 12 months, and 1 patient was treated for at least 2 years; these were all patients from Studies 201 and 202. (All patients in Study P1/2 were treated for 24 weeks and so were not included in the ≥6 months count). The duration of exposure is presented in Table 20.

**Table 20 Duration of Exposure**

Dosage	Number of patients exposed to the study drug				
	<3 months	≥ 3 to <6 months	≥ 6 months	≥ 12 months	≥24 months
40 mg/kg/wk	N=16	N=16	N=8	N=8	N=1*
80 mg/kg/wk	N=16	N=16	N=8	N=8	N=0

\*Note: With the 90-Day safety update, the total number of subjects with ≥24 months of exposure is 8 at the 40 mg/kg/week only

### 8.3.2 Relevant characteristics of the safety population:

There were 32 unique subjects in Study 201 and P1/2 combined. The demographic characteristics of both the studies combined are summarized in Table 21 and Table 22 and were similar for the two dose groups. Overall, 78% of the patients were ≥6 years of age. The patients in the US/Canada Study 201 were younger (4-10 years) and that in the Japanese Study P1/2 were

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older (5-16 years). All patients were ambulant in Study 201, whereas three patients were non-ambulant in Study P1/2. All patients in Study 201 were on steroids whereas in Study P1/2, being on steroid was not a requirement. Majority of the patient in both studies had exon 45-52 deletion.

**Table 21 Demographics and Baseline Characteristics of the Pooled Phase 2 studies (Safety Population)**

Parameter	Viltolarsen 40 mg/kg/wk (N=16)	Viltolarsen 80 mg/kg/wk (N=16)	Viltolarsen Total (N=32)
<b>Age (years)</b>			
Mean (SD)	7.6 (1.8)	7.5 (2.3)	7.5 (2.05)
Median	7.0	7.5	7.0
Minimum, maximum	4, 11	4, 12	4, 12
<b>Age group, n (%)</b>			
4 to <6 years	2 (12.5)	4 (25.0)	6 (18.8)
6 to <12 years	14 (87.5)	11 (68.8)	25 (78.1)
12 to 17 years	0	1 (6.3)	1 (3.1)
<b>Race, n (%)</b>			
White	8 (50.0)	7 (43.8)	15 (46.9)
Asian	8 (50.0)	9 (56.3)	17 (53.1)
Other	0	0	0
<b>Ethnicity, n (%)</b>			
Hispanic or Latino	0	1 (6.3)	1 (3.1)
Not Hispanic or Latino	8 (50.0)	6 (37.5)	14 (43.8)
Not reported	8 (50.0)	9 (56.3)	17 (53.1)
<b>Weight, kg</b>			
Mean (SD)	25.2 (7.8)	26.0 (9.6)	25.6 (8.6)
Median	23.9	23.8	23.9
Minimum, maximum	14.9, 41.7	15.5, 52.1	14.9, 52.1
<b>Height, cm</b>			
Mean (SD)	117.2 (11.2)	116.5 (11.0)	116.8 (10.9)
Median	114.8	117.5	115.8
Minimum, maximum	98.0, 140.7	99.4, 134.0	98.0, 140.7
<b>Body Mass Index (kg/m<sup>2</sup>)</b>			
Mean (SD)	18.0 (2.5)	18.6 (3.9)	18.3 (3.2)
Median	17.7	17.2	17.5
Minimum, maximum	14.2, 23.6	15.3, 30.2	14.2, 30.2

Source: Primary Reviewer Analysis of ADSL.xpt

**Table 22 Other Baseline Characteristics**

	Study 201 (N=16)	Study P1/2 (N=16)
<b>Exon Deletions, n(%)</b>		
43-52	0	0
45-52	7 (43.8)	6 (37.5)
47-52	1 (6.3)	0
48-52	3 (18.7)	3 (18.7)
49-52	3 (18.7)	2 (12.5)
50-52	2 (12.5)	2 (12.5)
52	0	3 (18.7)
<b>Ambulant</b>	<b>16 (100)</b>	<b>13 (81.2)</b>
<b>Steroid Use</b>	<b>16 (100)</b>	<b>14 (87.5)</b>
Deflazacort	12 (75)	0
Prednisolone/Prednisone	4 (25)	14 (100)

Source: Primary Reviewer Analysis of ADSL.xpt and ADCM.xpt

### 8.3.3 Adequacy of the safety database:

The safety database was mostly uncontrolled and small limiting the interpretation of any event being drug related.

## 8.4 Adequacy of Applicant's Clinical Safety Assessments

### 8.4.1 Issues Regarding Data Integrity and Submission Quality

Overall the safety database was adequate in format and quality for review. Given the orphan nature of the disease, the patient exposure appears adequate and generalizable to the US population.

### 8.4.2 Categorization of Adverse Events

Adverse events were coded according to the Medical Dictionary of Regulatory Activities (MedDRA) version 21.0 for reporting system organ class (SOC) and preferred term (PT) for study 201 and 201 and version 20.1 for Study P1/2. Only treatment-emergent AEs (TEAEs) were included in the safety analysis.

Treatment-emergent adverse events (TEAEs) were defined as any adverse event or worsening of an existing condition after initiation of the investigational product and through 30 days after

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completion of study participation (Study 201 and 202) or the post treatment observation period (14 days in Study P1/2). In counting the number of AEs reported, a continuous event (i.e., an AE reported more than once, and which did not cease) was counted only once with the worst-recorded severity; non-continuous AEs reported several times by the same patient were counted as multiple events. If a rollover patient had an AE that occurred in 2 studies (e.g., Study 201 and 202), the patient was counted only once when summarizing by patient, but the AE was counted as multiple events. Events present immediately prior to the first dose of study drug that did not worsen in severity, were not included. The investigators were asked to categorize adverse events as mild, moderate, or severe based on Common Terminology Criteria for Adverse Events (CTCAE) v 4.03 grades. In deriving the tabulation relating to the preferred term reporting, the severity of the recurrent AE was taken to be the most severe by the investigators. Investigators also assigned the causality of the AE as not related, possibly related and probably related. However, these had no bearings in the assessment AE frequencies.

The verbatim terms were manually reviewed for accuracy of coding. The applicant's coding resulted in appropriate translation of verbatim terms to preferred terms. However, AEs were often coded to multiple different equivalent Preferred Terms. The grouping of closely related terms or pooling of preferred terms was not accurately conducted by the applicant for a few preferred terms. These were recoded by the reviewer as shown in Table 23. The recoded dataset was used in the AE analyses summarized in the review.

**Table 23 Pooling of preferred Terms recoded by the Reviewer.**

Preferred Terms of the Applicant	Pooled Terms by the Reviewer
Upper respiratory tract infection, Nasopharyngitis, Sinusitis, Rhinorrhea, Rhinitis	Upper respiratory Tract infection
Catheter site swelling, Infusion site discomfort, Infusion site pain, Injection site bruising, Injection site erythema, Injection site extravasation, Injection site pain, Injection site reaction, Injection site swelling	Injection Site reaction
Abdominal pain, Abdominal pain upper	Abdominal Pain
Conjunctivitis, Conjunctivitis allergic	Conjunctivitis
Arthropod bite, Arthropod sting	Arthropod Bite
Dermatitis contact, Dermatitis,	Dermatitis
Foot fracture, Lower limb fracture	Fracture

### 8.4.3 Routine Clinical Tests

The routine clinical tests included anthropometrics, vital signs, hematology, chemistry, urinalysis, ECG, physical exam, cytokines, anti-dystrophin antibody and anti-viltolarsen

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antibody.

The reference range of some laboratory tests differed amongst laboratory tests. These were taken into consideration when evaluating outliers. The normal reference ranges were not provided for certain parameters. These have been pointed out in this review.

## 8.5 Safety Results

### 8.5.1 Deaths

There were no deaths reported in the application.

### 8.5.2 Serious Adverse Events

There were two serious AEs reported in the application. Study P1/2 and Study 202 each had 1 patient who experienced an SAE:

1. (b) (6) year old (b) (6) on viltolaren 80 mg/kg/wk with no other medical history and on concomitant prednisolone had serious Grade 2 upper respiratory tract infection that resolved in 3 weeks from time of onset. Patient's siblings had the same, therefore, the event appears unrelated to viltolarsen.
2. (b) (6) year old (b) (6) on 80 mg/kg/wk had a Grade lower limb fracture when jumping off a jungle gym. This event appears unrelated to viltolarsen.

### 8.5.3 Dropouts and/or Discontinuations Due to Adverse Effects

There were no dropouts/discontinuations due to adverse events reported in the application.

### 8.5.4 Significant Adverse Events

There were no significant adverse events reported in the application.

### 8.5.5 Treatment Emergent Adverse Events (TEAE) and Adverse Reactions

As discussed under Review Strategy the safety database was assessed in the following groups:

#### 1. Analysis of controlled safety database: duration of 4-weeks:

In a very short duration of 4 week of controlled safety data there was no clear dose related or treatment group related trend as shown in Table 24.

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**Table 24 TEAEs in the first 4 weeks of randomized treatment**

Treatment Emergent Adverse Event	40 mg/kg/wk (N=6)	80 mg/kg/wk (n=5)	Placebo (n=5)
Arthralgia	0	1 (20%)	1 (20%)
Dermatitis	1 (17%)	1 (20%)	0
Upper respiratory tract infection	0	1 (20%)	1 (20%)
Abnormal behavior	0	0	1 (20%)
Anxiety	1 (17%)	0	0
Attention deficit/hyperactivity disorder	1 (17%)	0	0
Contusion	0	1 (20%)	0
Cough	0	1 (20%)	0
Fall	0	0	1 (20%)
Headache	0	0	1 (20%)
Injection site reaction	1 (17%)	0	0
Nasal congestion	1 (17%)	0	0
Rash	1 (17%)	0	0
Respiratory tract congestion	0	1 (20%)	0

Source: Primary Reviewer Analysis

**2. Pooled analysis of Study 201 and P1/2: duration of 20-24 weeks:**

Study 201 and Study P1/2 both had 16 subjects each (8 at each dose). The study duration was 24 weeks, although in study 201, in the first 4 weeks subjects were randomized to the 2 doses of viltolarsen or placebo. The placebo patients were switched to either doses of viltolarsen after 4 weeks, hence this pool contributed to 20-24 week of safety from the two studies. TEAEs that occurred in  $\geq 10\%$  of the patients in either the 40 mg/kg/week or 80 mg/kg/week viltolarsen treatment group are presented in Table 25. The most frequent TEAEs were upper respiratory tract infection, cough, nasal congestion, pyrexia and injection site reaction and appeared dose related. The number of patients is too few to establish dose related trends in TEAEs, although there appeared no difference between doses.

**Table 25 TEAEs in  $\geq 10\%$  of the patients during 20-24 weeks of treatment with either 40 or 80 mg/kg/week viltolarsen (Pooled Studies 201 and P1/2)**

Treatment Emergent Adverse Event n (%)	40mg/kg/wk (n=16)	80 mg/kg/wk (n=16)
Upper respiratory tract infection	4 (25)	10 (62.5)
Injection site reaction	2 (12.5)	4 (25)
Contusion	3 (18.75)	2 (12.5)
Cough	2 (12.5)	3 (18.75)
Pyrexia	0 (0)	3 (18.75)

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Nasal congestion	3 (18.75)	0 (0)
Arthralgia	1 (6.25)	2 (12.5)
Dermatitis	2 (12.5)	1 (6.25)
Diarrhea	1 (6.25)	2 (12.5)
Influenza	2 (12.5)	1 (6.25)
Pain in extremity	2 (12.5)	1 (6.25)
Rash	2 (12.5)	1 (6.25)
Vomiting	1 (6.25)	2 (12.5)
Abdominal pain	0 (0)	2 (12.5)
Ejection fraction decreased	0 (0)	2 (12.5)
Urticaria	0 (0)	2 (12.5)

Source: Primary Reviewer Analysis

### TEAEs by Severity:

In study 201, 3 patients (19%) experienced moderate TEAE that included increased creatinine, potassium and BUN in Patient (b) (6) and vomiting in patient (b) (6) and pharyngitis in Patient (b) (6). In Study P1/2, 13 patients (81%) had at least one moderate TEAE, that included upper respiratory tract infection (7 patients), influenza, eczema, contusion and ejection fraction decreased (2 patients each) and urticaria (1 patient).

### **3. Long term safety analysis of Study 202: duration of 70-107 weeks based on a cut-off date of January 29, 2019 from the 144-week ongoing study:**

The 16 patients from Study 201 are enrolled in a long-term open label extension Study 202 at the same doses. A safety database cut-off of January 29, 2019 at the time of the application submission, provided an additional 70-107 weeks of safety data. The 90-day update with a cut-off of July 5, 2019 provided an additional 22 weeks of safety data. TEAEs that occurred during the long-term extension were similar to that observed in the 24 weeks of treatment period. Table 26 presents TEAEs that occurred in  $\geq 10\%$  of the patients in either the 40 mg/kg/week or 80 mg/kg/week viltolarsen or combined. No dose related trends were observed in the TEAEs, except for rash and injection site reaction that occurred in one additional patient at the 80 mg/kg/week dose. There are too few patients in each arm to draw any conclusions on dose related trends in TEAEs, although there appeared no difference between doses.

**Table 26 TEAEs in  $\geq 10\%$  of the patients during 70-107 weeks of treatment with either 40 or 80 mg/kg/week viltolarsen**

Treatment Emergent Adverse Event n(%)	40 mg/kg/wk (n=16)	80 mg/kg/wk (N=16)
Cough	5 (31.25)	3 (18.75)
Upper respiratory tract infection	4 (25)	3 (18.75)
Rash	2 (12.5)	3 (18.75)
Pyrexia	2 (12.5)	2 (12.5)

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Diarrhea	1 (6.25)	2 (12.5)
Influenza	2 (12.5)	1 (6.25)
Injection site reaction	1 (6.25)	2 (12.5)
Nasal congestion	2 (12.5)	1 (6.25)
Vomiting	2 (12.5)	1 (6.25)
Body tinea	2 (12.5)	0 (0)
Limb injury	0 (0)	2 (12.5)
Respiratory tract congestion	2 (12.5)	0 (0)

### 8.5.6 Laboratory Findings

#### Hematology:

Small changes from baseline were observed in most hematology parameters in Study 201 and 202. None of the parameters showed dose dependent changes from baseline. No clinically meaningful trends were observed.

**Study 201 and 202:** No TEAE associated with hematology parameters were observed.

I looked for hematology parameters that would signal anemia as in the 12-week proof of concept Study DMT01 in DMD patients (at doses  $\leq 20$  mg/kg/wk), 7 cases of anemia were observed (discussed below). No clinically meaningful changes in hematology parameters were observed.

**Study P1/2:** One patient (P1/2- (b) (6)) at 40 mg/kg/week had decreased hemoglobin and hematocrit at the first visit (week 2) from normal to low that stayed low throughout until week 26. It worsened by greater than  $>10$  g/L from his own baseline. Hematocrit followed the same time course as hemoglobin (not reported in Table 27). Decreased hemoglobin, hematocrit and ferritin was also reported as a TEAE for this patient. These were considered secondary to blood loss due to repetitive blood sampling by the investigator. No other clinically meaningful signal was observed in the other subjects. No dose related decrease in hemoglobin after dosing was observed in Study P1/2.

**Table 27 Decrease in hemoglobin and report of TEAE in Study P1/2**

Subject	Study Day	Observed Hemoglobin (Reference range 117 -145 g/L)	TEAE
P1/2- (b) (6) (Study P1/2) 40 mg/kg/week	Baseline	131	Decreased hemoglobin, hematocrit and ferritin
	Week 2	114	
	Week 3	117	
	Week 4	117	
	Week 5	114	

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	Week 7	115	
	Week 9	114	
	Week 11	117	
	Week 13	112	
	Week 15	121	
	Week 17	115	
	Week 19	113	
	Week 21	118	
	Week 23	120	
	Week 25	104	
	Week 26	109	

**Study DMT01:** 7 patients reported anemia that included all cohorts (1.25 mg/kg, 5 mg mg/kg and 20 mg/kg). The decrease in hemoglobin, hematocrit and leucocytes occurred within the first week of dosing in all cohorts. It resolved with a couple days in Cohorts 1 and 2. However, 3 patients in cohort 3, had decreases in hemoglobin and hematocrit that lasted for longer duration of the study as shown in Table 28 and TEAE was listed as non-resolved. Hematocrit followed the same trend in these subjects (not shown in Table 28). The applicant explains these differences as large amount of blood collection intended for close monitoring in the First-in-human study. It appeared that the highest dose cohort had decreases for longer duration and a TEAE of anemia was reported unresolved. A clear signal attributing the decrease in hemoglobin to viltolarsen cannot be made at this time.

**Table 28 Decrease in hemoglobin and report of TEAE in Study DMT101**

Subject And Dose	Study Day	Observed Hemoglobin (Reference range 13.5-17 g/dL)	TEAE
(b) (6) (Study DMT01)  20 mg/kg/week	Day 0	12.4	Anemia
	Day 1	11.5	Not resolved
	Day 2	11.2	
	Day 3	11.0	
	Day 4	10.5	
	Day 5	10.6	
	Day6	10.4	
	Day 7	10.4	
	Day 14	11.0	
	Day 21	11.3	
	Day 28	11.7	
	Day 42	11.8	

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	Day 56	11.4	
	Day 70	11.6	
	Day 77	12.1	
	Day 84	11.5	
	Day 97	12.1	
	Day 104	10.8	
(b) (6) (Study DMT01)  20 mg/kg/week	Day 0	13.6	Anemia
	Day 7	11.6	
	Day 14	12.2	Not resolved
	Day 21	12.4	
	Day 28	13.2	
	Day 42	13.0	
	Day 56	12.2	
	Day 70	11.9	
	Day 77	11.8	
	Day 84	12.8	
	Day 97	11.9	
	Day 104	11.4	
(b) (6) (Study DMT01)  20 mg/kg/week	Day 0	14.2	Anemia
	Day 1	13.2	
	Day 2	12.3	Not resolved
	Day 3	13.0	
	Day 4	12.4	
	Day 5	12.0	
	Day 6	11.5	
	Day 7	12.0	
	Day 14	11.6	
	Day 21	11.8	
	Day 28	11.7	
	Day 42	11.8	
	Day 56	11.6	
	Day 70	11.7	
	Day 77	11.8	
	Day 84	11.3	
	Day 97	10.7	
	Day 104	11.3	

Clinical meaningfulness of findings is unclear as no trends were observed at higher doses in Study 201/202.

Urine Analysis:

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Urine analysis parameters included: albumin, amorphous phosphate and urate crystals, creatinine, erythrocytes, leucocytes, glucose hyaline casts, alpha-1 microglobulin, creatinine, N-acetyl-beta-D-glucosaminidase, occult blood, osmolality, protein, specific gravity and urobilinogen. No clinically meaningful trends were observed in urine analyses and no dose dependent changes were observed in any parameters. Two sporadic cases of trace occult blood were observed in Studies 201 and 202. Additional discussion on renal parameters are elaborated under Section 8.6, Analysis of submission specific AE of interest "Kidney Function".

### Clinical Chemistry:

No clinically significant changes from baseline or trends were observed in any clinical chemistry parameters in Study 201 and 202.

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were high at baseline (2-25 times ULN), as expected in the DMD population. Given the elevations of AST and ALT that can be observed in the DMD population, bilirubin and gamma glutamyl transferase (GGT) may be more informative markers of liver injury. No patient had bilirubin values that shifted from normal or low to a high value in any study. Only one patient (b) (6) had a sporadic >ULN serum alkaline phosphate at Week 37 of 318 U/L (Reference range 93-308 U/L), where prior and subsequent values were within the normal limits. No values of GGT were above the ULN in any study. Therefore, there is no indication of Drug-induced liver injury in the safety database.

Creatine kinase (CK) and Lactate dehydrogenase (LDH) was also high in all studies, as expected in DMD patients. It interesting to note that CK showed a trend of reduction in Studies 201 and 202, however, reductions of similar magnitude were not observed in Study P1/2. The reasons for this appear unclear. No patient had CK that worsened from baseline.

No increase in Cystatin C from low or normal to high was observed in any study.

In Study 201, blood creatinine increased, blood potassium increased, and blood urea increased for 1 patient (b) (6) 80 mg/kg/wk on Day 85. The events were all reported as TEAE and was termed as verbatim term: "Elevated BUN of 30". These events resolved 9 days later with no intervention. The events were assessed as moderate in severity. Relevant clinical laboratory assessments are also further discussed under Section 8.6, Analysis of submission specific AEs of interest "Kidney Function".

No TEAEs were associated with laboratory findings for ALT, AST, CK or Cystatin C.

### **8.5.7 Vital Signs**

I analyzed the vital signs including temperature, heart rate, respiratory rate, diastolic, systolic blood pressure, weight, height and body mass index. Vital signs were measured pre-infusion, and at 1 hour (at end of infusion) and 2 hours post-dose (and at 6 hours if the 2-hour collection

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showed significant change from pre-dose) at various visits in Study 201 and 202. Vital signs were measured pre-infusion, and at 2 hours after the end of infusion (and at 6 hours if the 2-hour collection showed significant change from pre-dose) in Study P1/2.

While outliers were observed in many patients, these were marginal deviation from normal ranges and were generally sporadic in nature and occurred at baseline as well. There were no systematic clinical meaningful trends observed for any vital signs. Only pyrexia associated with vital signs was reported as an TEAE.

### 8.5.8 Electrocardiograms (ECGs)

The following observations were made by the Reviewers in the Interdisciplinary Review Team (IRT) for Cardiac safety [Drs. Girish Bende and Christine Garnett (Lead)]:

**Study 201:** 12-lead ECGs were performed after the patient had rested for 10 minutes in the supine position at screening, Day 1, Week 13, and Week 25/ET. Relatively small mean changes from baseline were observed for PR, QRS, QT, QTcB, QTcF, and RR at Weeks 13 and 25. No patient had a QTcF value higher than 432 msec or a change from baseline of >33 msec. None of these changes from baseline were considered to be clinically meaningful. No patient had a cardiac rhythm or interpretation value at Week 13 or 25 that was considered abnormal-clinically significant by local cardiologist.

**Study 202:** 12-lead ECGs are scheduled at Weeks 25, 37, 49, 73, 97, 121, 146, and 169 (or early termination) and were performed as described for Study 201. For the Week 96 data cut, no patient had a QTcF value higher than 434 msec or a change from Week 25 of >46 msec. None of these changes from baseline were considered to be clinically meaningful. No patient had a cardiac rhythm or interpretation value that was considered abnormal clinically significant by the local cardiologist.

**Study P1/2:** Intensive ECGs were performed on Days 1 and 162 at 60, 40, and 20 minutes before the start of infusion; 30 minutes after the start of infusion; immediately after the end of infusion; and 1, 2, and 4 hours after the end of infusion. Electrocardiography was performed by the sponsor, and the ECG parameters were measured by the central ECG laboratory.

On standard ECG, no clinically meaningful abnormal findings were observed in either group. The worst post-treatment QTcF interval was  $\leq 450$  msec and worst post-treatment QTcB interval was  $\leq 480$  msec in all subjects. In addition, the change from baseline to the worst value after treatment was  $\leq 30$  msec QTcF and QTcB.

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**Table 29 Summary of significant abnormal resting standard 12-lead ECG (post treatment value)**

Item	Treatment group	N	Worst value after treatment							
			≤450 msec		450 < ≤480 msec		480 < ≤500 msec		500 msec <	
			n	%	n	%	n	%	n	%
QTc interval (Fridericia correction)	All patients	16	16	100.0	0	0.0	0	0.0	0	0.0
	40 mg/kg group	8	8	100.0	0	0.0	0	0.0	0	0.0
	80 mg/kg group	8	8	100.0	0	0.0	0	0.0	0	0.0
QTc interval (Bazett correction)	All patients	16	9	56.3	7	43.8	0	0.0	0	0.0
	40 mg/kg group	8	5	62.5	3	37.5	0	0.0	0	0.0
	80 mg/kg group	8	4	50.0	4	50.0	0	0.0	0	0.0

Source: Clinical Study Report NS065/NCNP01-P1/2

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14.3.6.13 Resting 12-Lead Electrocardiogram

Change in Descriptive Statistics Values

Item	Treatment Group / Visit / Timepoint	N	Mean (SD)	Median	Min~Max	Difference		
						Mean (SD)	Median	Min~Max
All Patients	V1a / 1 hr pre-dose	16	399.6 (23.2)	404	352~438	-	-	-
	V1a / 40 min pre-dose	16	397.9 (22.4)	399	350~432	-	-	-
	V1a / 20 min pre-dose	16	402.6 (22.5)	406	366~443	-	-	-
	V1a / Average of 3 pre-dose	16	400.1 (22.4)	403.5	356~438	-	-	-
	V1a / 30 min after the start	16	404.9 (21.5)	410.5	363~435	4.8 (6.1)	4.5	-3~17
	V1a / 0 hr post-dose	16	405.7 (21.8)	407.5	358~444	5.6 (6.3)	4	-4~19
	V1a / 1 hr post-dose	16	405.1 (20.1)	407	366~445	4.9 (8.3)	7	-15~17
	V1a / 2 hrs post-dose	16	402.1 (19.1)	402.5	366~439	1.9 (8.1)	4	-14~11
	V1a / 4 hrs post-dose	16	401.4 (17.3)	403	365~430	1.3 (10.2)	4	-16~19
	V24 / 1 hr pre-dose	16	394.1 (17.6)	397.5	352~419	-6.0 (11.7)	-4	-28~ 9
	V24 / 40 min pre-dose	15	394.3 (20.0)	396	347~426	-7.9 (8.5)	-7	-27~ 4
	V24 / 20 min pre-dose	16	394.6 (20.3)	398.5	347~430	-5.5 (8.9)	-6	-19~ 8
	V24 / 30 min after the start	16	399.2 (19.2)	398	352~432	-0.9 (11.4)	-1	-24~14
	V24 / 0 hr post-dose	16	402.3 (20.5)	403.5	349~438	2.2 (9.8)	2	-16~17
	V24 / 1 hr post-dose	16	397.9 (20.6)	399.5	354~434	-2.3 (10.6)	-2	-19~13
	V24 / 2 hrs post-dose	16	394.7 (17.2)	393	362~436	-5.4 (10.7)	-5	-25~ 8
V24 / 4 hrs post-dose	16	398.6 (16.5)	399.5	364~435	-1.6 (10.7)	-0.5	-23~13	
QTc Interval Fridrichia's Correction (msoc)	40 mg/kg	8	399.4 (28.0)	408.5	352~438	-	-	-
	V1a / 40 min pre-dose	8	399.0 (29.2)	411.5	350~432	-	-	-
	V1a / 20 min pre-dose	8	404.8 (26.8)	411.5	366~443	-	-	-
	V1a / Average of 3 pre-dose	8	401.1 (27.8)	411	356~438	-	-	-
	V1a / 30 min after the start	8	404.6 (26.1)	418	363~435	3.5 (5.1)	3	-3~11
	V1a / 0 hr post-dose	8	405.0 (28.4)	414.5	358~444	3.9 (4.8)	2.5	-4~12
	V1a / 1 hr post-dose	8	403.4 (26.1)	404.5	366~445	2.3 (8.9)	5.5	-15~10
	V1a / 2 hrs post-dose	8	400.6 (24.6)	401	366~439	-0.5 (7.9)	1.5	-12~10
	V1a / 4 hrs post-dose	8	400.1 (22.1)	399	365~430	-1.0 (9.9)	-0.5	-15~ 9
	V24 / 1 hr pre-dose	8	390.6 (21.3)	391.5	352~419	-10.5 (12.5)	-11	-28~ 9
	V24 / 40 min pre-dose	8	391.8 (25.9)	393	347~426	-0.4 (8.6)	-8.5	-27~ 3
	V24 / 20 min pre-dose	8	392.4 (25.0)	398	347~430	-8.8 (5.8)	-8.5	-17~ 0
	V24 / 30 min after the start	8	396.4 (24.6)	395.5	352~432	-4.8 (11.1)	-3.5	-24~ 9
	V24 / 0 hr post-dose	8	399.0 (26.9)	402.5	349~438	-2.1 (8.6)	-1.5	-16~10
	V24 / 1 hr post-dose	8	397.5 (26.7)	399	354~434	-3.6 (9.7)	-5	-17~11
	V24 / 2 hrs post-dose	8	393.8 (21.9)	393	362~436	-7.4 (12.0)	-7	-25~ 6
V24 / 4 hrs post-dose	8	397.3 (22.0)	395	364~435	-3.9 (10.7)	-5	-23~ 8	
80 mg/kg	V1a / 1 hr pre-dose	8	399.9 (19.0)	400.5	365~425	-	-	-
	V1a / 40 min pre-dose	8	396.8 (14.9)	398	373~419	-	-	-
	V1a / 20 min pre-dose	8	400.5 (18.9)	400.5	372~434	-	-	-
	V1a / Average of 3 pre-dose	8	399.1 (17.3)	400.5	370~426	-	-	-
	V1a / 30 min after the start	8	405.3 (17.5)	404.5	381~432	6.1 (7.0)	5.5	-3~17
	V1a / 0 hr post-dose	8	406.4 (14.6)	405.5	389~430	7.3 (7.5)	4.5	-2~19
	V1a / 1 hr post-dose	8	406.8 (13.3)	410.5	385~424	7.6 (7.1)	8	-2~17
	V1a / 2 hrs post-dose	8	403.5 (13.1)	405.5	381~422	4.4 (8.1)	7.5	-14~11
	V1a / 4 hrs post-dose	8	402.8 (12.3)	405	388~424	3.6 (10.7)	4	-16~19
	V24 / 1 hr pre-dose	8	397.6 (13.4)	400	369~412	-1.5 (9.6)	1	-22~ 8
	V24 / 40 min pre-dose	7	397.1 (11.4)	396	384~413	-6.1 (8.6)	-2	-22~ 4
	V24 / 20 min pre-dose	8	396.9 (14.7)	399	374~416	-2.3 (10.6)	2.5	-19~ 8
	V24 / 30 min after the start	8	402.0 (12.9)	402.5	379~420	2.9 (11.0)	7	-19~14
	V24 / 0 hr post-dose	8	405.6 (12.3)	403.5	385~424	6.5 (9.4)	9.5	-9~17
	V24 / 1 hr post-dose	8	398.3 (14.0)	399.5	368~417	-0.9 (11.9)	0.5	-19~13
	V24 / 2 hrs post-dose	8	395.6 (12.2)	393.5	377~412	-3.5 (9.7)	-5	-22~ 8
V24 / 4 hrs post-dose	8	399.9 (9.7)	402.5	383~410	0.8 (10.9)	2.5	-21~13	

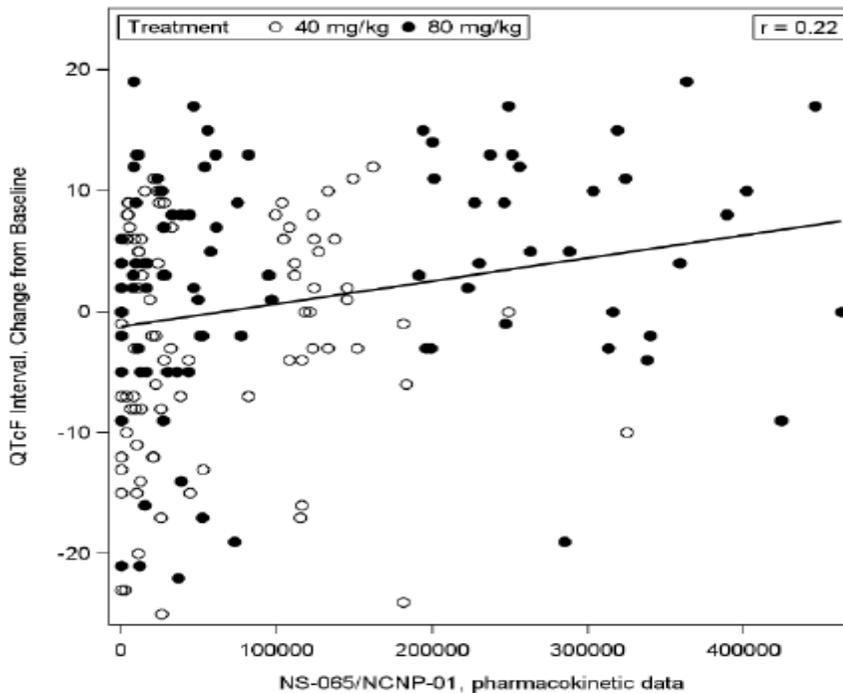
Source: Clinical Study Report NS065/NCNP01-P1/2

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**Figure 15 Change from Baseline in Resting 12-Lead Electrocardiogram QTcF Values and Blood Drug Concentration (naïve pooled analysis)**



Source: Clinical Study Report NS065/NCNP01-P1/2

*IRT Reviewer's comment: Overall, the ECGs collected in Study NS065/NCNP01 do not show large mean increases in QTc according to the sponsor's analysis. However, the study evaluated limited number of subjects at the maximum therapeutic dose (80 mg/kg/wk; n=8)*

### 8.5.9 QT

The applicant did not conduct a thorough QT study for viltolarsen. The applicant requested a waiver for a thorough QT study based on the severe nature of Duchenne muscular dystrophy (DMD), the unmet medical need, and the extreme difficulty in recruiting patients for such studies.

*IRT Reviewer's comment:*

*While the existing nonclinical and clinical data do not suggest a concerning proarrhythmic risk for viltolarsen, the data are not adequate for the characterization of drug effect on the QTc interval. In the Phase 1/2 study, the ECGs were collected on Day 1 and Day 162 at 60, 40, and 20 min before the start of infusion, at 30 minutes and immediately after end of infusion, and 1, 2, and 4 hours after the end of infusion. However, the study evaluated limited number of subjects at the maximum therapeutic dose (80 mg/kg/wk; n=8). In addition, the ECGs collected in other Phase-2 studies (Studies # NS-065/NCNP-01-201 and 202) were also not adequate. The ECG data*

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*are available from only few subjects receiving the therapeutic dose (80 mg/kg/wk) and these data were not collected at the end-of-infusion (Tmax).*

The ongoing Confirmatory Study will provide additional QTc data on viltolarsen. Recommendations for characterizing the effects of viltolarsen on the QTc interval to exclude large increases in QTc (>20 msec) were made to the applicant and should be completed as a post marketing requirement. The applicant's request to waive the Thorough QTc study can be made upon review of the results of this Confirmatory Study.

### **8.5.10 Cytokines**

No clinically meaningful changes in Interleukin-6, Monocyte Chemoattractant Protein-1, or Tumor necrosis factor were observed.

### **8.5.11 Anti-Dystrophin Antibody**

Anti-dystrophin antibody was detected in 1 patient (# [REDACTED]<sup>(b) (6)</sup>) at 80 mg/kg/week dose at week 13 and 24. The amount of dystrophin mRNA or protein produced remained unaffected. Please also refer to immunogenicity review of the application.

### **8.5.12 Anti-Viltolarsen Antibody**

Anti-viltolarsen antibody was negative in all patients. Please also refer to immunogenicity review of the application.

## **8.6 Analysis of Submission-Specific Safety Issues**

### **8.6.1 Injection Site Reactions**

Viltolarsen was infused once per week over a 1-hour infusion period. Application of a topical or local anesthetic was an option prior to placement of the IV catheter. The following injection related reactions were observed, all of which were recoded by the reviewer as 'Injection Site Reactions' in the Studies 201, 202 and P1/2 as shown in Table 30. Each of these TEAEs were mild in severity and were all resolved on the day of onset. These TEAEs occurred at any time during the study as seen in Table 30.

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**Table 30 Injection related reactions AE terms in the Safety Population**

Study	Sponsor's AEDECOD	AE Start Day	AE End Day	AE Severity	Dose
201					
	Infusion site discomfort	30	30	Mild	40 mg/kg/wk
	Infusion site bruising*	161	161	Mild	80 mg/kg/wk
	Infusion site pain	8	8	Mild	40 mg/kg/wk
	Injection site reaction*	98	98	Mild	80 mg/kg/wk
202					
	Catheter site swelling	252	252	Mild	80 mg/kg/wk
	Infusion site pain	173	173	Mild	40 mg/kg/wk
	Infusion site extravasation	180	180	Mild	80 mg/kg/wk
P1/2					
	Injection site erythema	8	8	Mild	80 mg/kg/wk
	Infusion site pain	37	37	Mild	80 mg/kg/wk
	Injection site swelling	80	80	Mild	80 mg/kg/wk

\*These occurred in the same patient

No infection related to infusion site or ports were reported in any study. Overall, mild injection site reactions occurred that resolved the same day.

In conclusion, no Warnings or Precautions are warranted in the Product Label at this time.

### 8.6.2 Kidney Function

The kidney has been the major site of antisense oligonucleotide deposition in non-clinical studies. Accumulation is generally dose and duration dependent and can lead to degenerative tubular changes. Such pattern of nephrotoxicity was also observed in viltolarsen nonclinical program. Due to limitations in monitoring renal function with creatinine, the applicant included additional markers of kidney function [creatinine, Cystatin-C] and urinary function [of beta-N-acetyl-D-glucosaminidase (NAG), protein, albumin,  $\alpha$ 1-microglobulin,  $\beta$ 2-microglobulin] in their development. Increases in NAG were observed in the development program without clinically significant changes in Cystatin-C. A consult was requested from the Division of Cardiology and Nephrology (DCN) (Drs. Kirtida Mistry, Kimberly Smith and Aliza Thompson) to provide assessment on the viltolarsen renal safety seen in the development program and the utility of Urine Beta-N-acetyl-D-glucosaminidase levels to assess tubular damage in DMD patients. This section summarizes our conclusions from Drs. Mistry, Smith and Thompson.

*Nonclinical observations:*

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Non-clinical studies of viltolarsen showed evidence of tubular toxicity but no glomerular toxicity. In a 26-week study in mice, animals developed elevations in urea, creatinine, and cystatin C, and two males died of nephrotoxicity. Histopathology showed vacuolation and deposition of basophilic material in the epithelium and dilation of distal tubules and/or collecting ducts, vacuolation of the epithelium of the proximal tubules, and fibrosis. In addition, masses and/or thickening of the ureter were seen in three mice on macroscopic examination, which were shown to be transitional cell carcinomas on histopathology. In a 12-week study in monkeys, histopathology showed epithelial vacuolation and basophilic changes in proximal tubules and mononuclear cell infiltration and edema in the medullary interstitium. In a 39-week study in monkeys, histopathology showed “very slight” dilatation of the renal tubules, “very slight” epithelial vacuolation, and “very slight” basophilic changes in the proximal tubules. There were no associated changes in serum creatinine. Biomarkers of renal tubular injury were not collected in preclinical studies.

Exposures ( $C_{max}$  and/or  $AUC_{0-24h}$ ) of viltolarsen in humans receiving 80 mg/kg/week in studies 201 and P1/P2 provided a 1.4X safety margin the NOAEL for the renal findings.

### *Renal Monitoring in viltolarsen program:*

In Study 201/202, serum creatinine, serum cystatin C, and a random urine sample for dipstick urinalysis, microscopy, protein, albumin, beta-N-acetyl-D-glucosaminidase (NAG),  $\alpha$ 1-microglobulin, and creatinine were collected at screening, every 2 weeks from Weeks 3 through 9, monthly through Week 49, then every 12 weeks. In addition, a 24-hour urine was collected at screening, Day 1, and Week 24 for protein, albumin, NAG,  $\beta$ 2-microglobulin,  $\alpha$ 1-microglobulin, creatinine, electrolytes, and uric acid for Study 201 only.

For Study P1/2, serum creatinine and cystatin C were assessed at screening, weekly through Week 5, then every 2 weeks. A random urine for dipstick urinalysis, protein, albumin, NAG,  $\alpha$ 1-microglobulin, and creatinine was obtained weekly. In addition, a 24-hour urine was collected at baseline, Week 12, Week 24, and Week 25 for protein, albumin, NAG,  $\beta$ 2-microglobulin,  $\alpha$ 1-microglobulin, creatinine, electrolytes, and uric acid.

There were no specified renal adverse events of special interest or renal-related stopping criteria.

### *Renal Safety Findings in Pooled studies 201/202 and P1/2:*

There were no deaths or AEs leading to treatment discontinuation or dose reduction. The following patients had renal-related AEs reported during the study:

Subject (b) (6) (b) (6) year-old on 80 mg/kg/week in Study 201 had adverse events of “blood creatinine increased,” “blood potassium increased,” and “blood urea increased” reported on

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day 85 that resolved by day 93 without a change in viltolarsen dosing. Creatinine was 0.2 mg/dL from screening through Week 9 then increased to 1.7 mg/dL at the next check on Week 13. BUN increased from 21 mg/dL to 30 mg/dL, and potassium increased from 4.8 mmol/L to 5.8 mmol/L from screening to Week 13. Labs were rechecked at an unscheduled visit two weeks later, and creatinine had returned to 0.3 mg/dL and remained between 0.2 and 0.3 mg/dL for the rest of the study. Of note, cystatin C was 0.7 mg/dL at both baseline and Week 13. The investigator reported that the event was not related to study drug. The same patient had an AE of “hypercalciuria” (reported term: urine calcium crystals) on Day 336 during the extension study, which was reported as resolved on Day 343 without interruption of viltolarsen dosing.

*DCN Reviewer’s comment: We were unable to locate any details regarding the clinical circumstances surrounding the elevated serum creatinine value, if there were any, but note that the cystatin C data suggest it could have been a lab error or non-renal related increase. The findings were transient and fully resolved despite continued treatment with viltolarsen, making a drug-related renal toxicity unlikely.*

Subject (b) (6) year-old on 40 mg/kg/week in Study P1/2 had adverse event of beta-N-acetyl-D-glucosaminidase increased. The patient also had reported events of albumin urine present,  $\alpha$ 1-microglobulin increased, blood urine present, and protein urine present reported on the same date. The events were assessed as unrelated to drug and related to “running a 800-meter race.” The events were reported as recovered one week later without a change in viltolarsen dose.

Subject (b) (6) year-old on 80 mg/kg/week in Study P1/2 had adverse event of beta-N-acetyl-D-glucosaminidase increased that was reported as recovered after 2 weeks without a change in viltolarsen dosing.

Subject (b) (6) year-old on 80 mg/kg/week in P1/2 with three adverse events of beta-N-acetyl-D-glucosaminidase increased reported that each resolved within one week without a change in viltolarsen dosing. The patient also had an adverse event of  $\beta$ 2-microglobulin urine increased that was reported as recovered 1 week after the end of the viltolarsen treatment regimen.

*DCN Reviewer’s comment: All three patients had transient increases in urinary biomarkers that were not normalized to urine concentration and resolved despite continuation of treatment with viltolarsen. The clinical significance of these findings is not clear.*

### *Analyses of Markers of Kidney Function:*

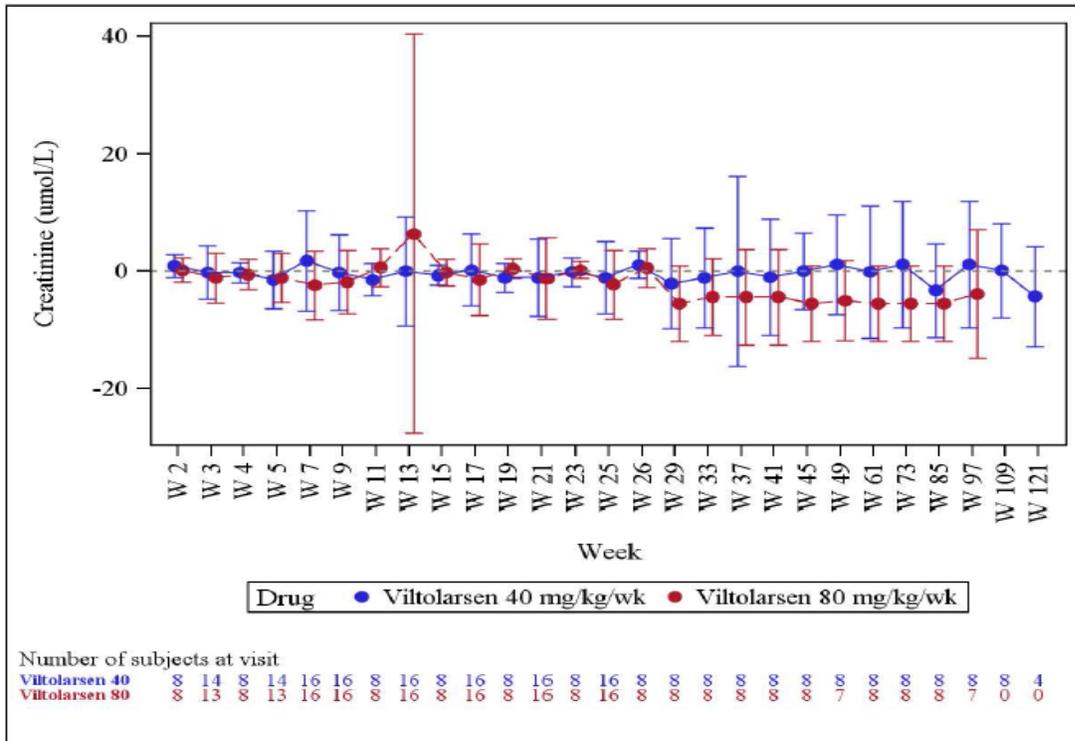
There was no obvious difference in mean change from baseline in serum creatinine over time by treatment arm (Figure 16).

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**Figure 16: Mean change from baseline in serum creatinine over time (pooled phase 2 studies)**



Source: Applicant, Safety update report Figure 14.3.2.1.2.1.

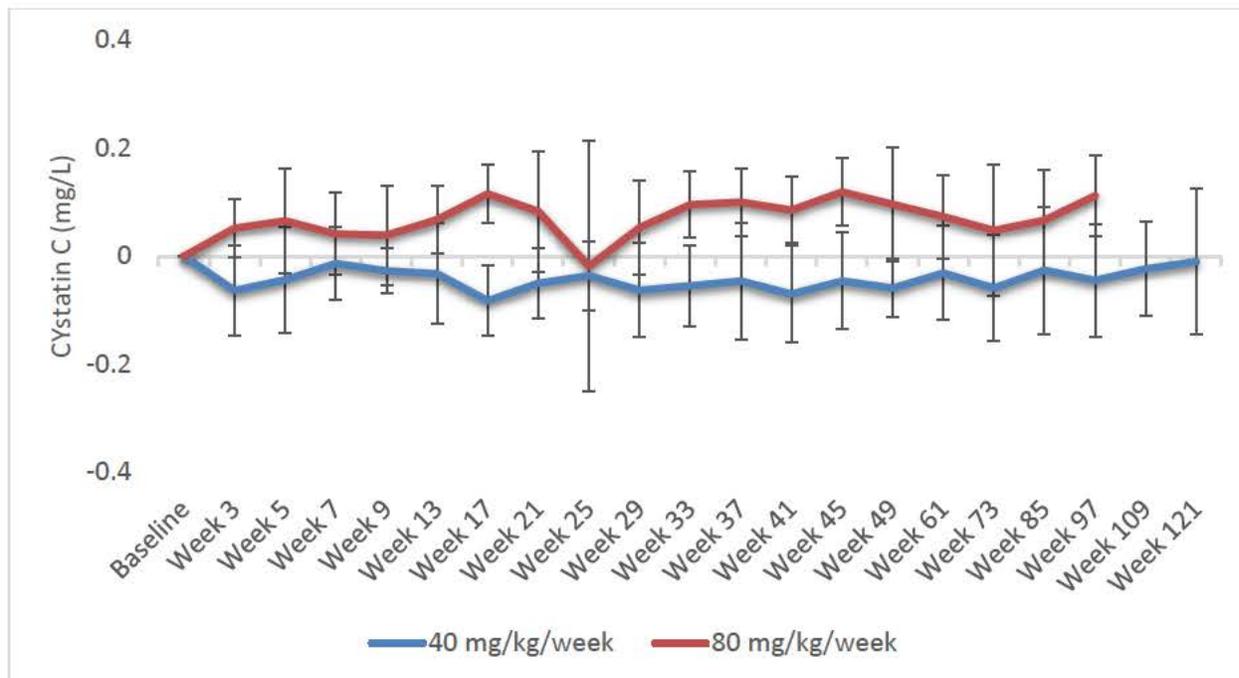
Mean changes in cystatin C in study 201/202 are shown by treatment arm in the Figure 17. There appears to be a small numerical decrease in mean cystatin C in the 40 mg/kg/week arm and small numerical increase in mean cystatin C in the 80 mg/kg/week arm, both observed early and maintained over time. A similar pattern was not seen in Study P1/2 (Figure 18). Review of individual patient cystatin C values for Study 201/202 (Figure 19) or serum creatinine and cystatin C data by study (see Appendix) also did not raise concern.

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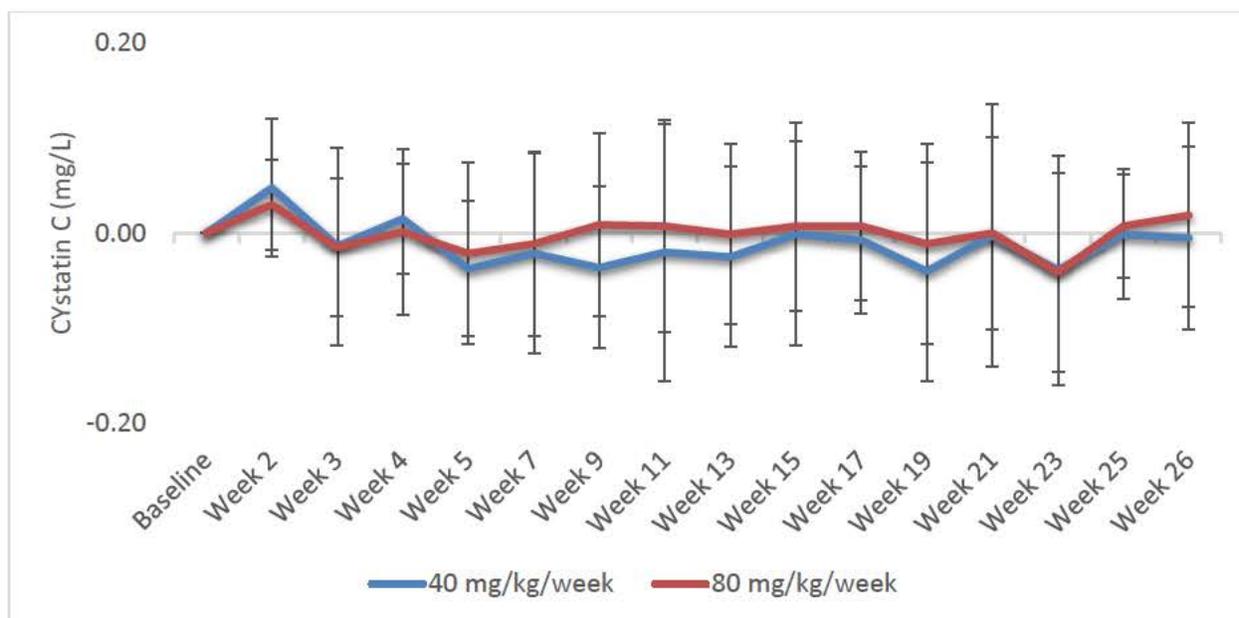
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**Figure 17: Mean change from baseline in serum cystatin C over time in Study 201/202 (top) and Study P1/2 (bottom)**



Source: Primary reviewer's analyses.

**Figure 18: Mean change from baseline in serum cystatin C over time in Study P1/2**

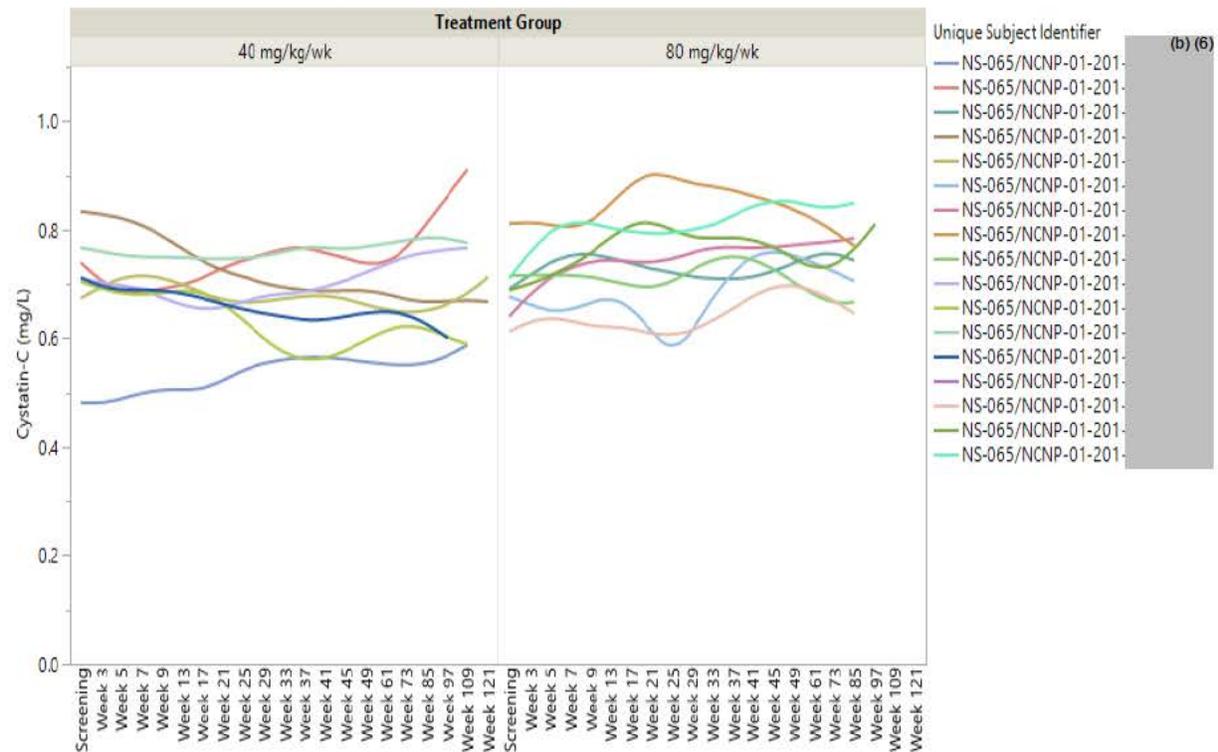


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Source: Primary reviewer's analyses.

Figure 19: Individual patient cystatin C values over time by treatment arm in Study 201/202



Source: Primary reviewer's analyses.

Analyses of Urinary Biomarkers:

Several urinary biomarkers of renal tubular toxicity were measured in studies 201/202 and P1/2 (protein, albumin, NAG,  $\alpha$ 1-microglobulin,  $\beta$ 2-microglobulin. The applicant identified seven patients with a shift in NAG level from normal to high (defined as over 11.5 U/L; baseline values ranged from 0.35 to 5.3 U/L) during study P1/2, including the three patients with related AEs noted above. Three of the patients had only one high value during the trial ( (b) (6) and one had intermittent elevated values that normalized despite continued treatment (b) (6). Three patients, all in the 80 mg/kg/week group ( (b) (6) ), had multiple elevated values that persisted through the end of the study. None of these patients had other findings suggestive of renal injury. Two additional patients with elevations in NAG to  $\geq$ 11.5 U/L was identified during Study 201/202, both in the 80 mg/kg/week group.

There were no consistent patterns or increases across biomarkers.

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### *DCN Reviewer's comment:*

*Beta-N-acetyl-D-glucosaminidase (NAG) is an enzyme expressed in the proximal tubules of the kidney and is one of several exploratory urinary biomarkers of kidney injury. Urinary NAG and other urinary biomarkers have been qualified by the FDA as part of a safety biomarker panel to aid in the detection of kidney tubular injury in phase 1 trials in healthy volunteers (<https://www.fda.gov/drugs/cder-biomarker-qualification-program/reviews-qualification-biomarker-clusterin-clu-cystatin-c-cy5c-kidney-injury-molecule-1-kim-1-n>). Excretion of NAG into the urine correlates with proximal tubular cell injury and, in studies of various renal diseases including acute kidney injury and treatment with nephrotoxic compounds, increased urinary NAG levels have typically been observed before increases in serum creatinine.*

*In the viltolarsen studies, intermittent elevations in urinary NAG above a specified threshold were noted in some patients without other findings suggestive of renal toxicity. The clinical significance of the observed changes and the utility of NAG in detecting tubular damage, both in this population and in general, is unclear. It is also not clear why cutoffs of 11.5 U/L was selected for the analyses by the applicant.*

### *Renal Safety Findings in Study DMT01*

In the Phase 1 Study DMT01 conducted in Japan, adverse events of beta-N-acetyl-D-glucosaminidase increased were reported for 9 of 10 patients, "protein urine present" for 8 of 10 patients, and "albumin urine present" for 7 of 10 patients. Other renal-related AEs included beta 2-microglobulin increased and cystatin C increased in two patients each and hematuria, blood urine present, and urine protein/creatinine ratio increased in one patient each. All but one "protein urine present" event were assessed as CTCAE Grade 1.

On further investigation of the urine protein findings, the applicant concluded that viltolarsen interferes with the pyrogallol red dye-binding method of 24-hour urine protein measurement and, using an alternative assay (Coomassie brilliant blue), levels were within the normal range. This informed the assays used in later studies in the development program (i.e., Studies 201/202 and P1/2). The applicant observed, however, that this would not explain the other events reported in this study, which seemed out of proportion to the numbers seen in other viltolarsen studies and the fact that lower viltolarsen doses were administered in DMT01 (i.e., the highest dose administered was 20 mg/kg/week).

*Reviewer's comment: The significance of these findings is unclear.*

Overall, there is no obvious signal for renal toxicity based on either laboratory data or renal-related adverse events at the time of this review, though the safety database is limited in size and duration. The applicant is conducting a 48-week placebo-controlled confirmatory study that

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will include extensive renal monitoring appropriate for the DMD population which will inform future labeling regarding effects of viltolarsen on renal function.

In conclusion, although no clinically interpretable renal toxicity was observed in a small number of patients in clinical studies, Warnings or Precautions for monitoring renal function should be warranted in the Product Label based on the renal toxicity observed in animals and the nature of renal toxicity observed in animals being similar to that of the approved antisense oligonucleotide golidersen (VYONDYS 53®) that includes warning and precautions for monitoring renal function in the product label.

### 8.6.3 Drug Hypersensitivity

I (VT) look for adverse events that related to drug hypersensitivity as drug hypersensitivity was listed under Warnings and Precautions in a similar approved antisense oligonucleotide golidersen (VYONDYS 53®). Given the limitation of the open label safety database of viltolarsen, I looked for a dose effect for adverse events that likely could be related to drug hypersensitivity to evaluate the possibility of drug related hypersensitivity. I evaluated these in the same grouping as that the overall safety analysis, i.e. (1) pooling 24-week studies 201 and P1/2 shown in Table 31a and Table 31b and (2) extension study 202 for exposures >24 weeks shown in Table 31c. In the safety database only one preferred term “drug hypersensitivity” was found in the 40 mg/kg/week dose on the first day of dosing. The verbatim term was ‘contact allergic reaction to Tegaderm’ and therefore does not appear drug related. Other terms that I looked for included dermatitis, rash, urticaria, pruritis, cough and pyrexia. Most events were mild, except or one event of dermatitis at 40 mg/kg/week dose (patient (b) (6)) and one event was urticaria at 80 mg/kg/week dose (patient (b) (6)) which was of moderate severity. Most cases of rash were a single event in a patient that resolved within 1-10 days from onset, except for the moderate case of dermatitis that took 29 days to resolve. The patient with urticaria was reported to have urticaria once in every month that resolved in a day. This patient had no reported event of urticaria in the open label extension study.

#### Table 31 Incidence of hypersensitivity related terms

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(a) Pooled Study 201 and P1/2 for 24 weeks

AE Term	40 mg/kg/wk	80 mg/kg/wk
Cough	2	3
Dermatitis	1	1
Drug hypersensitivity	1	0
Pyrexia	0	3
Rash	2	1
Urticaria	0	2

(b) Pooled Study 201 and P1/2; combining dermatitis and rash terms as Rash

AE Term	40 mg/kg/wk	80 mg/kg/wk
Cough	2	3
Drug hypersensitivity	1	0
Pyrexia	0	3
Rash	2	2
Urticaria	0	2

(c) Study 202 for >24 weeks

AE Term	40 mg/kg/wk	80 mg/kg/wk
Cough	5	3
Erythema	1	0
Pyrexia	2	2
Rash	2	4

Based on the small safety database and no dose correlation to these events, attribution to any of these events to hypersensitivity to viltolarsen cannot be established at this time.

### 8.6.4 Off-target effects: Hypopigmentation

Antisense oligonucleotides can cause sequence-dependent side effects if there is a sequence that is homologous to the unintended target among native mRNA or pre-mRNA sequences. In vitro gene expression studies in cultured human cells indicated the potential for drug mediated changes in mRNA levels for several targeted genes. One of these genes APCDD1 is associated with hereditary hypotrichosis that may be of human relevance. Mutation in this gene can slow or stop hair growth with light-colored or hypopigmented hair shafts. In Study P1/2, one patient had a TEAE of 'hair depigmented'. The event started on Day 95 and lasted 108 days. Amino acid tests showed no clinically meaningful observations with respect to blood concentrations of melanin precursors. The applicant regards the relationship of this hair color change with off-target activity as unclear since hypotrichosis was not observed.

Therefore, at this time there is no clear evidence that the hair color change is drug related.

## 8.7 Safety Analyses by Demographic Subgroups

### Age

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Out of 32 patients in the safety analysis, 6 (18%) of the patients were between the ages 4 to <6, 25 (78%) patients were of the ages 6 to <12 years, and 1 (4%) patient was >12 years. The number of patients in each age group are very small for any meaningful comparisons.

### **Sex:**

All patients in the study were males.

### **Race:**

Out of 32 patients in the safety analysis, 15 (47%) of the patients were white, 17 (53%) were Asians. No obvious difference was observed.

## **8.8 Specific Safety Studies/Clinical Trials**

No special safety studies were performed.

## **8.9 Additional Safety Explorations**

### **8.9.1 Human Carcinogenicity or Tumor Development**

No neoplasms were reported in the Application.

### **8.9.2 Human Reproduction and Pregnancy**

Viltolarsen is to be administered to males only. There are no human data available on the use of viltolarsen in pregnancy or milk production.

### **8.9.3 Pediatrics and Assessment of Effects on Growth**

The safety database was open label, hence effects on growth cannot be assessed.

### **8.9.4 Overdose, Drug Abuse Potential, Withdrawal, and Rebound**

No case of overdose was observed. The potential for drug abuse appears negligible. No studies examining withdrawal or rebound were conducted.

## **8.10 Safety in the Postmarket Setting**

### **8.10.1 Safety Concerns Identified Through Postmarket Experience**

No post marketing experience is included in the application.

### **8.10.2 Expectations on Safety in the Postmarket Setting**

Because of the small sample size of the patients, it is difficult to predict the safety profile of

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viltolarsen.

### **8.10.3 Additional Safety Issues From Other Disciplines**

Please refer to section 4 of this review.

## **8.11 Integrated Assessment of Safety**

An integrated assessment of safety was not performed given the small sample size of the safety database.

## **9 Advisory Committee Meeting and Other External Consultations**

---

An Advisory Committee meeting was not held for viltolarsen.

## **10 Labeling Recommendations**

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### **10.2 Prescription Drug Labeling**

Edits to the label are proposed in a separate labeling review with Review Team.

## **11 Risk Evaluation and Mitigation Strategies (REMS)**

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No REMS are recommended for viltolarsen.

## **12 Postmarketing Requirements and Commitments**

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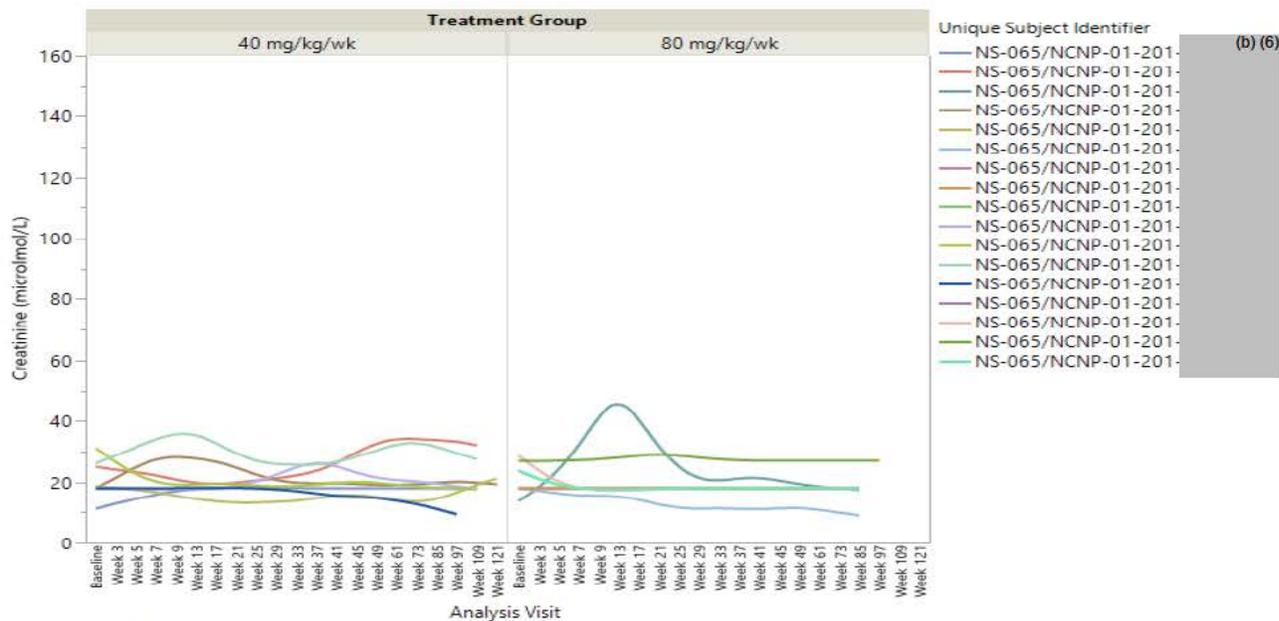
- A confirmatory study to verify the clinical benefit of viltolarsen will be conducted.
- Collection of ECG assessments in the confirmatory study will be included to support the applicant's request to waive a thorough QT study. If these data do not support a TQT study waiver, the applicant will need to evaluate the effect of viltolarsen on the QTc interval in a dedicated study as per the ICH E14 guideline.

## 13 Appendices

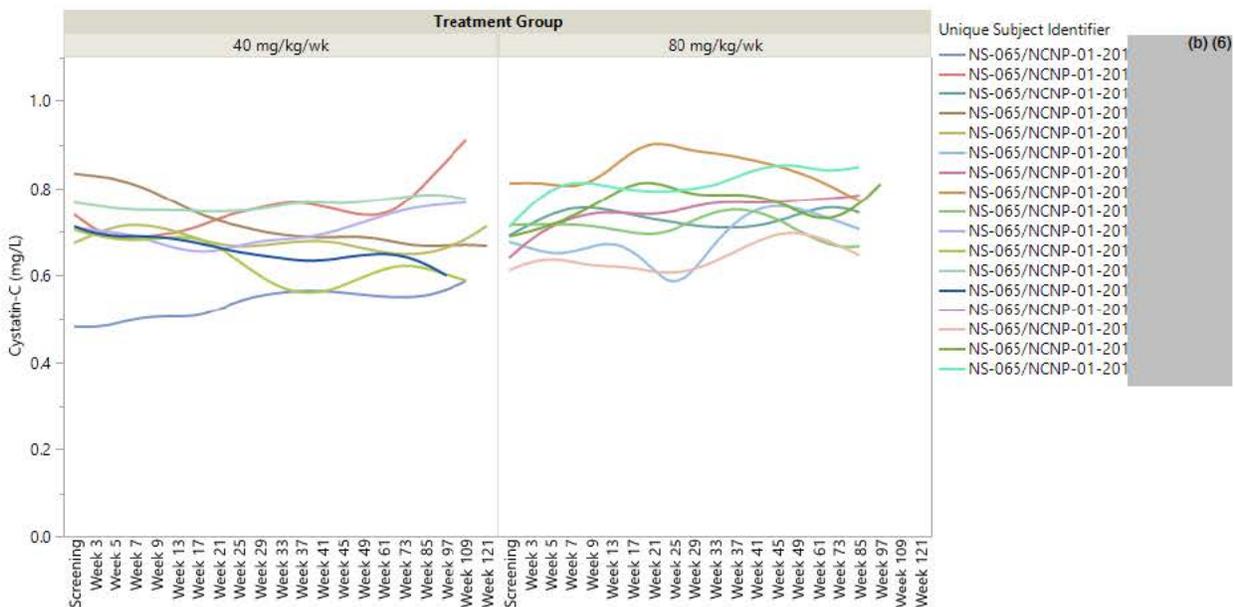
### 13.1 Individual patient creatinine and cystatin C data by study.

#### Study 201/202

#### Creatinine



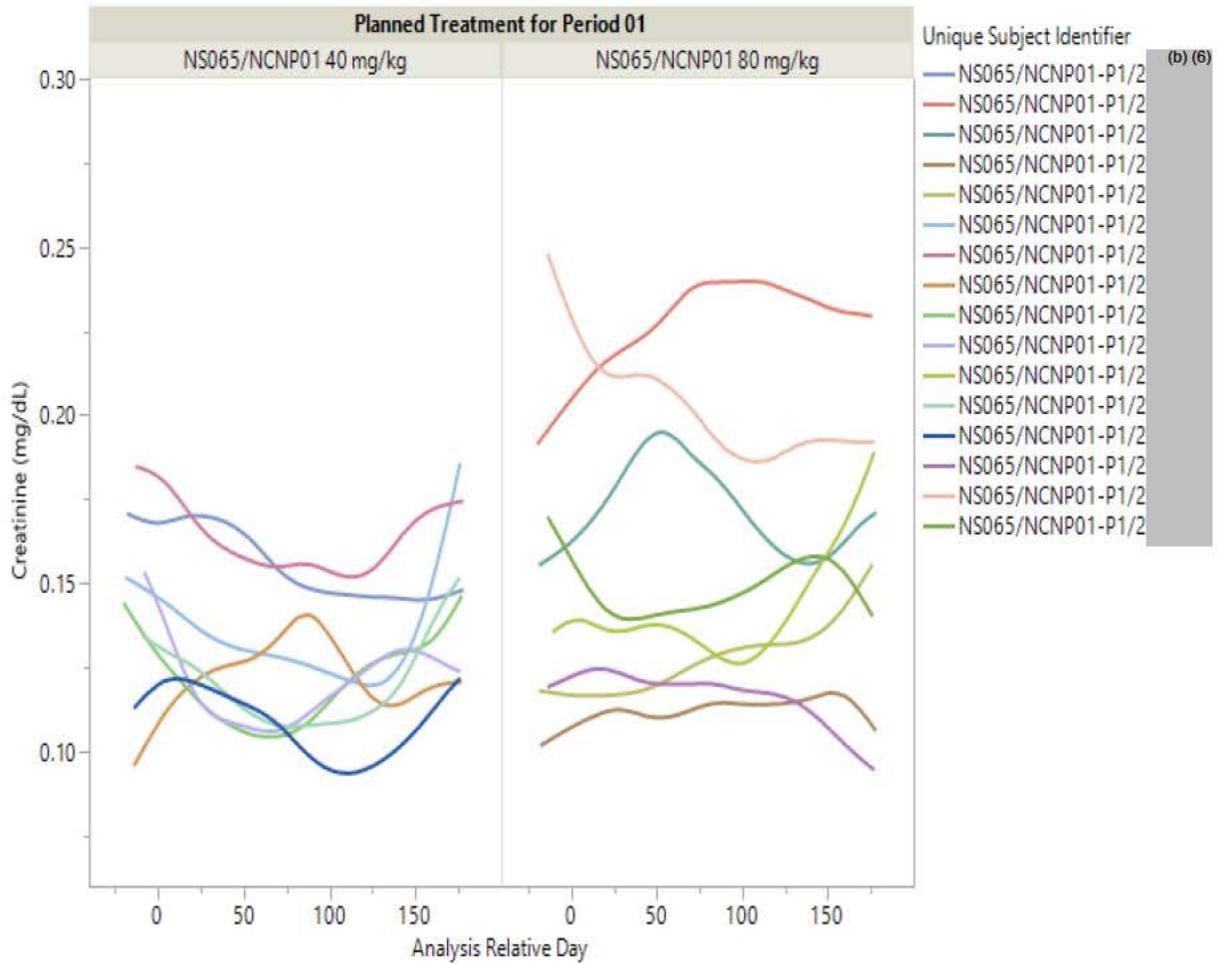
#### Cystatin C



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**Study P1/2**  
**Creatinine**

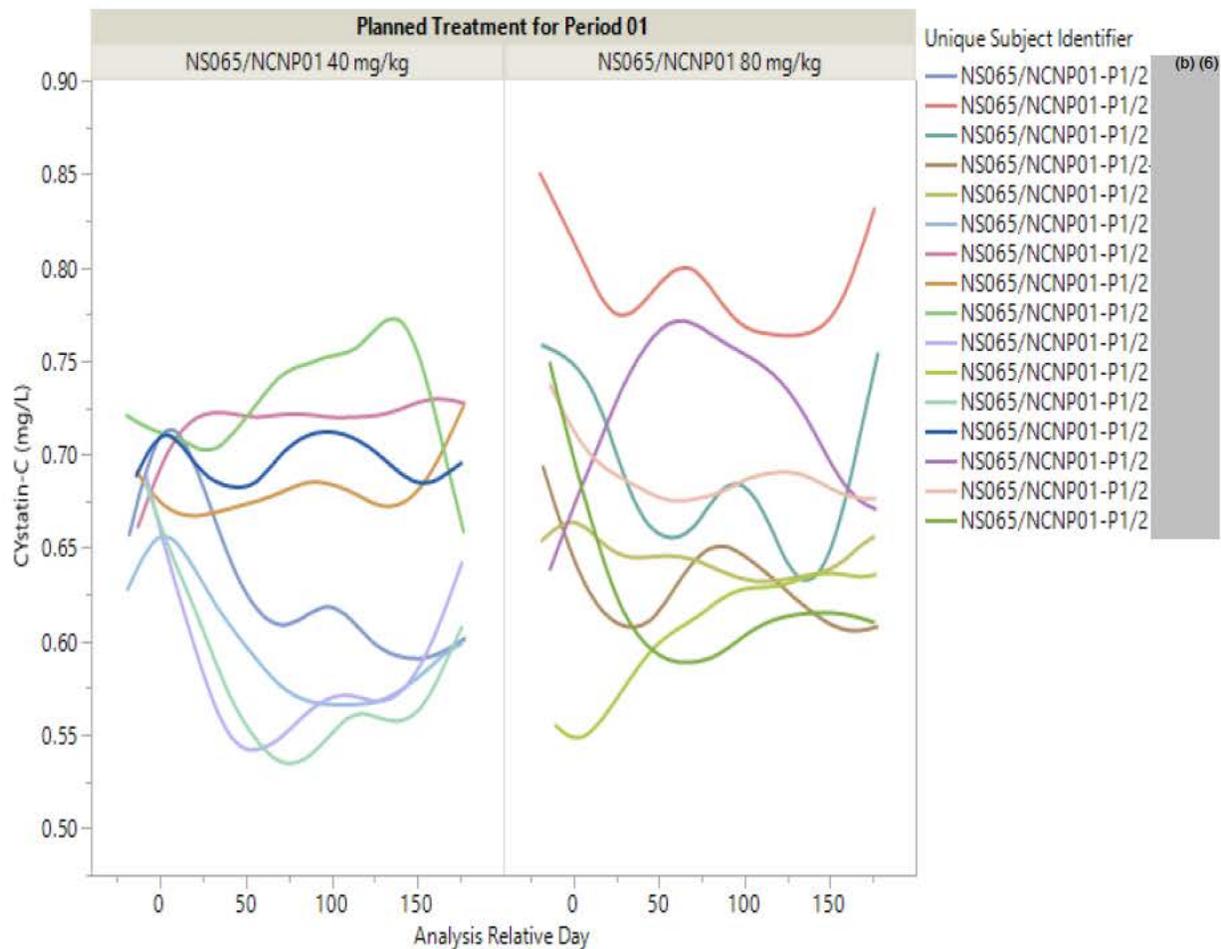


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Cystatin C

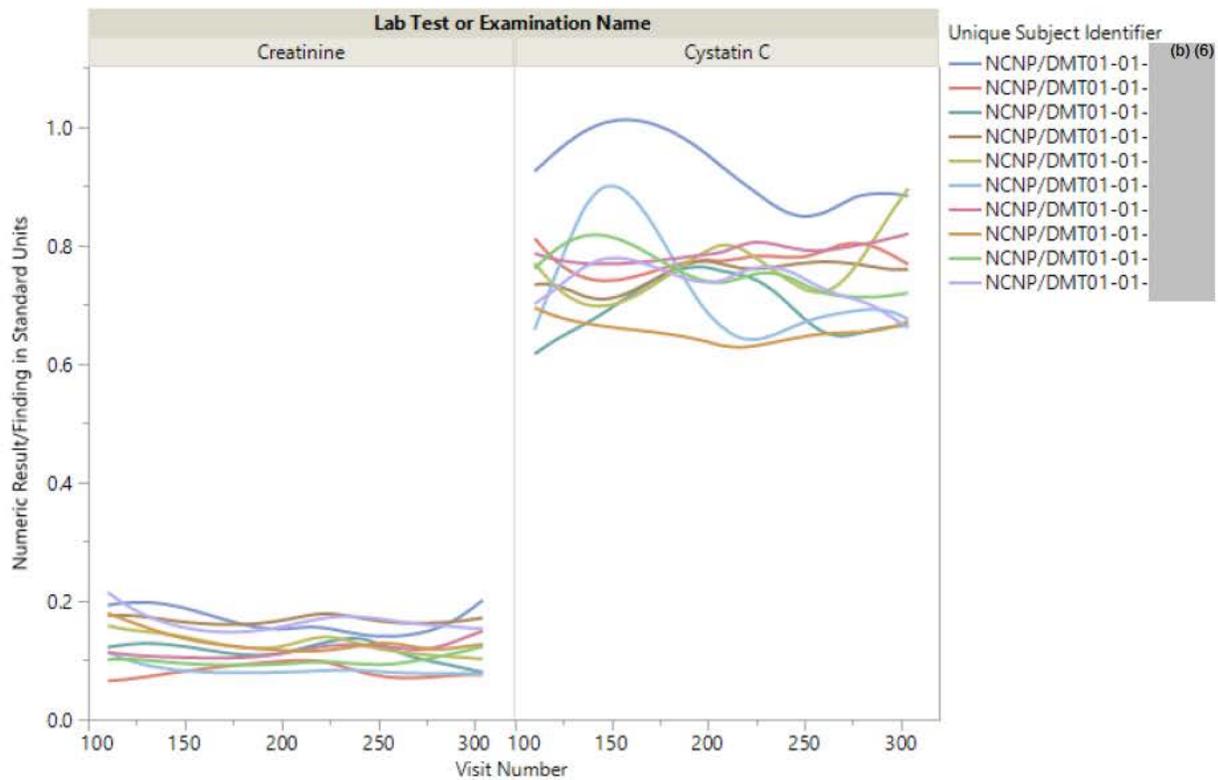


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**Study DMT101**

**Creatinine and Cystatin C**



**13.2 Financial Disclosure**

**Covered Clinical Study (Name and/or Number):**

Was a list of clinical investigators provided:	Yes <input checked="" type="checkbox"/>	No <input type="checkbox"/> (Request list from Applicant)
Total number of investigators identified: <u>7</u>		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): <u>0</u>		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): <u>0</u>		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR		

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Viltolarsen, NDA 212154

54.2(a), (b), (c) and (f):		
Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: _____		
Significant payments of other sorts: _____		
Proprietary interest in the product tested held by investigator: _____		
Significant equity interest held by investigator in S		
Sponsor of covered study: _____		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes <input type="checkbox"/>	No <input type="checkbox"/> (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes <input type="checkbox"/>	No <input type="checkbox"/> (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) _____		
Is an attachment provided with the reason:	Yes <input type="checkbox"/>	No <input type="checkbox"/> (Request explanation from Applicant)

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/s/  
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VERNEETA TANDON  
08/11/2020 10:06:38 AM

BAIKUNTHA ARYAL  
08/12/2020 08:06:08 AM

ASHUTOSH V RAO  
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I concur with the statistical review.

HSIEN MING J HUNG  
08/12/2020 08:36:44 AM

TERESA J BURACCHIO  
08/12/2020 08:39:33 AM

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**Date:** May 13, 2020  
**From:** Kirtida Mistry, Physician, Division of Cardiology and Nephrology  
**Through:** Kimberly Smith, Team Leader  
Aliza Thompson, Deputy Director  
Division of Cardiology and Nephrology  
**To:** Annie Nguyen, Regulatory Project Manager, Division of Neurology 1  
**Subject:** Renal toxicity and safety monitoring of viltolarsen

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### **Background**

Viltolarsen is an antisense oligonucleotide (ASO) that binds to a specific sequence in exon 53 of the dystrophin pre-mRNA transcript and blocks exon/intron splicing, leading to mRNA transcripts that lack exon 53. On December 12, 2019, the Division of Neurology 1 received an original NDA for viltolarsen injection for the treatment of Duchenne muscular dystrophy (DMD) in patients who have a confirmed mutation of the DMD gene that is amenable to exon 53 skipping.

DMD is the most severe of the muscular dystrophies. It is inherited as an X-linked recessive trait, and therefore primarily affects males. Mutations result in an absence of functional dystrophin, a protein complex which maintains muscle integrity, leading to necrosis of muscle fibers. DMD manifests clinically at 2 to 3 years of age with progressive muscle weakness and cardiomyopathy, and patients often die in the second or third decade of life from respiratory and/or cardiac failure. For patients with amenable mutations, it is thought that skipping exon 53 will produce a shorter but functional dystrophin and a milder phenotype.

The applicant has applied for accelerated approval based on muscle dystrophin expression as a surrogate endpoint and plans to confirm the treatment benefit in the post-marketing setting based on assessments of strength and function. No patients have been randomized in the confirmatory trial. Of note, similar ASO drugs, eteplirsen and golodirsen were granted accelerated approval in 2016 and 2019 for the treatment of patients with DMD and dystrophin mutations amenable to exon 51 and 53 skipping, respectively.

Renal toxicity has been described in preclinical and clinical studies of ASOs, including elevations in serum creatinine, proteinuria, acute kidney injury, and acute glomerulonephritis. Clinical toxicities appear to be drug-specific and are not always well-correlated with preclinical findings. The Division of Neurology 1 has asked the Division of Cardiology and Nephrology to 1) comment on the renal safety of viltolarsen as seen in the development program, 2) provide labeling recommendations regarding renal toxicity with viltolarsen and need for monitoring, 3) comment on the utility of Urine Beta-N-acetyl-D-glucosaminidase levels to assess tubular damage in DMD patients, and 4) advise on the adequacy of the renal monitoring in the protocol for confirmatory study NS-065/NCNP-01-301, a 48-week study in exon 53 skippable DMD patients.

## Materials Reviewed<sup>1</sup>

1. Summary of Clinical Safety submitted September 30, 2019 and Safety Update submitted December 31, 2019
2. Protocols NS-065/NCNP-01-201 (Amendment 6 dated November 28, 2017), NS-065/NCNP-01-202 (Amendment 7 dated June 24, 2018), NS065/NCNP01-P1/2 (Version 2 dated June 16, 2016), NCNP/DMT01 (Version 12 dated September 28, 2017), and NS-065/NCNP-01-301 (Amendment 3 dated March 2, 2020)
3. Emails from Dr. Veneeta Tandon dated March 16, April 7, and April 16, 2020 containing analyses of trial data
4. Nonclinical overview

## Non-Clinical Pharmacology/Toxicology of ASOs and Viltolarsen

The kidney is a major site of ASO deposition in non-clinical studies. Early histologic changes include basophilic granules and vacuoles in renal tubular epithelial cells that are usually reversible and not considered adverse in the absence of signs of degeneration/regeneration or impaired kidney function. Accumulation is generally dose and duration dependent and can lead to degenerative tubular changes. Some ASOs (e.g., drisapersen) also cause an immune-mediated glomerulonephritis in preclinical studies. The pattern of nephrotoxicity varies between ASOs, likely due to backbone chemistry and sequences of individual agents, although this is not well-understood.

Non-clinical studies of viltolarsen showed evidence of tubular toxicity but no glomerular toxicity. In a 26-week study in mice, animals developed elevations in urea, creatinine, and cystatin C, and two males died of nephrotoxicity. Histopathology showed vacuolation and deposition of basophilic material in the epithelium and dilation of distal tubules and/or collecting ducts, vacuolation of the epithelium of the proximal tubules, and fibrosis. In addition, masses and/or thickening of the ureter were seen in three mice on macroscopic examination, which were shown to be transitional cell carcinomas on histopathology. In a 12-week study in monkeys, histopathology showed epithelial vacuolation and basophilic changes in proximal tubules and mononuclear cell infiltration and edema in the medullary interstitium. In a 39-week study in monkeys, histopathology showed “very slight” dilatation of the renal tubules, “very slight” epithelial vacuolation, and “very slight” basophilic changes in the proximal tubules. There were no associated changes in serum creatinine. Biomarkers of renal tubular injury were not collected in preclinical studies.

Exposures ( $C_{max}$  and/or  $AUC_{0-24h}$ ) of viltolarsen in humans receiving 80 mg/kg/week in studies 201 and P1/P2 were higher than those parameters in mice and monkeys at the NOAEL in toxicity and safety pharmacology studies. The NOAEL was based, in part, on histopathologic changes in the kidney and bladder.

## Overview of Design of Viltolarsen Clinical Studies

The NDA submission includes data from the following clinical studies:

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<sup>1</sup> NDA 212154. \\cdsesub1\evsprod\nda212154\

- *NS-065/NCNP-01-201 (Study 201)*: randomized, placebo-controlled, pivotal study in 16 boys 4 to <10 years of age with DMD conducted in the U.S. and Canada. During phase 1, two cohorts of 8 patients each were randomized 3:1 to 40 mg/kg/week viltolarsen or placebo (cohort 1) or 80 mg/kg/week viltolarsen or placebo (cohort 2). After 4 weeks, all patients switched to open-label viltolarsen for an additional 20 weeks. All 16 patients completed this study and continued the same dose in an ongoing open-label extension study NS-065/NCNP-01-202 (Study 202).
- *NS065/NCNP01-P1/2 (Study P1/2)*: completed, open-label study in 16 male patients  $\geq 5$  and <18 years of age with DMD conducted in Japan. Two groups of 8 participants each received viltolarsen 40 mg/kg or 80 mg/kg weekly for 24 weeks.
- *NCNP/DMT01 (Study DMT01)*: completed, open-label study in 10 male patients 5 to <18 years of age with DMD conducted in Japan. Patients were treated with viltolarsen 1.25 (n=3), 5 (n=3), or 20 (n=4) mg/kg weekly for 12 weeks.

Proposed labeling includes only the 80 mg/kg/week dose tested in Studies 201/202 and P1/2, and, as such, those studies will be the primary focus of this review. The applicant provided data both by individual study and pooled across studies 201/202 and P2/2 (“pooled phase 2 studies”).

#### *Renal Eligibility Criteria*

Study 201 required that laboratory test results be “within the normal range at the Screening visit, or if abnormal, are not clinical significant.”

Study P1/2 excluded patients with “severe hepatic or renal disease precluding participation in this study in the opinion of the investigator.”

#### *Renal Monitoring*

In Study 201/202, serum creatinine, serum cystatin C, and a random urine sample for dipstick urinalysis, microscopy, protein, albumin, beta-N-acetyl-D-glucosaminidase (NAG),  $\alpha 1$ -microglobulin, and creatinine were collected at screening, every 2 weeks from Weeks 3 through 9, monthly through Week 49, then every 12 weeks. In addition, a 24-hour urine was collected at screening, Day 1, and Week 24 for protein, albumin, NAG,  $\beta 2$ -microglobulin,  $\alpha 1$ -microglobulin, creatinine, electrolytes, and uric acid.

For Study P1/2, serum creatinine and cystatin C were assessed at screening, weekly through Week 5, then every 2 weeks. A random urine for dipstick urinalysis, protein, albumin, NAG,  $\alpha 1$ -microglobulin, and creatinine was obtained weekly. In addition, a 24-hour urine was collected at baseline, Week 12, Week 24, and Week 25 for protein, albumin, NAG,  $\beta 2$ -microglobulin,  $\alpha 1$ -microglobulin, creatinine, electrolytes, and uric acid.

There were no specified renal adverse events of special interest or renal-related stopping criteria.

## Renal Safety Findings – Pooled Phase 2 Studies

### *Exposure:*

A total of 32 patients were exposed to viltolarsen for a mean of 12 months in the pooled phase 2 studies. Of these, the 16 enrolled in Study P1/2 were treated for 24 weeks, per protocol.

### *Baseline Characteristics:*

All patients were male with a mean age of 7.5 years (range 4 to 12). The population was approximately half white (15 [47%]) and half Asian (17 [53%]). Baseline serum creatinine values ranged from ~0.1 to 0.3 mg/dL and cystatin C from ~0.6 to 0.9 mg/L.

### *Adverse Events:*

There were no deaths or AEs leading to treatment discontinuation or dose reduction. The following patients had renal-related AEs reported during the study:

Subject (b) (6) year-old on 80 mg/kg/week in Study 201 had adverse events of “blood creatinine increased,” “blood potassium increased,” and “blood urea increased” reported on day 85 that resolved by day 93 without a change in viltolarsen dosing. Creatinine was 0.2 mg/dL from screening through Week 9 then increased to 1.7 mg/dL at the next check on Week 13. BUN increased from 21 mg/dL to 30 mg/dL, and potassium increased from 4.8 mmol/L to 5.8 mmol/L from screening to Week 13. Labs were rechecked at an unscheduled visit two weeks later, and creatinine had returned to 0.3 mg/dL and remained between 0.2 and 0.3 mg/dL for the rest of the study. Of note, cystatin C was 0.7 mg/dL at both baseline and Week 13. The investigator reported that the event was not related to study drug. The same patient had an AE of “hypercalciuria” (reported term: urine calcium crystals) on Day 336 during the extension study, which was reported as resolved on Day 343 without interruption of viltolarsen dosing.

*Reviewer’s comment: We were unable to locate any details regarding the clinical circumstances surrounding the elevated serum creatinine value, if there were any, but note that the cystatin C data suggest it could have been a lab error or non-renal related increase. The findings were transient and fully resolved despite continued treatment with viltolarsen, making a drug-related renal toxicity unlikely.*

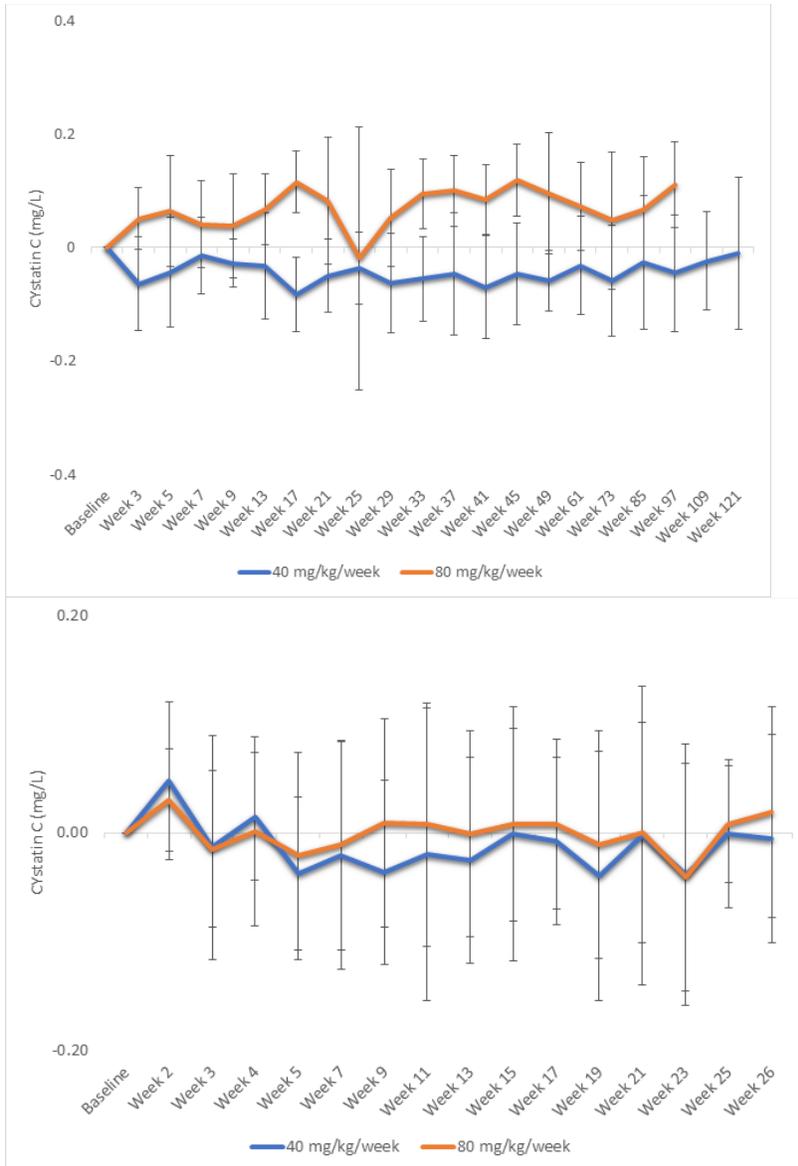
Subject (b) (6) year-old on 40 mg/kg/week in Study P1/2 had adverse event of beta-N-acetyl-D-glucosaminidase increased. The patient also had reported events of albumin urine present,  $\alpha$ 1-microglobulin increased, blood urine present, and protein urine present reported on the same date. The events were assessed as unrelated to drug and related to “running a 800-meter race.” The events were reported as recovered one week later without a change in viltolarsen dose.

Subject (b) (6) year-old on 80 mg/kg/week in Study P1/2 had adverse event of beta-N-acetyl-D-glucosaminidase increased that was reported as recovered after 2 weeks without a change in viltolarsen dosing.





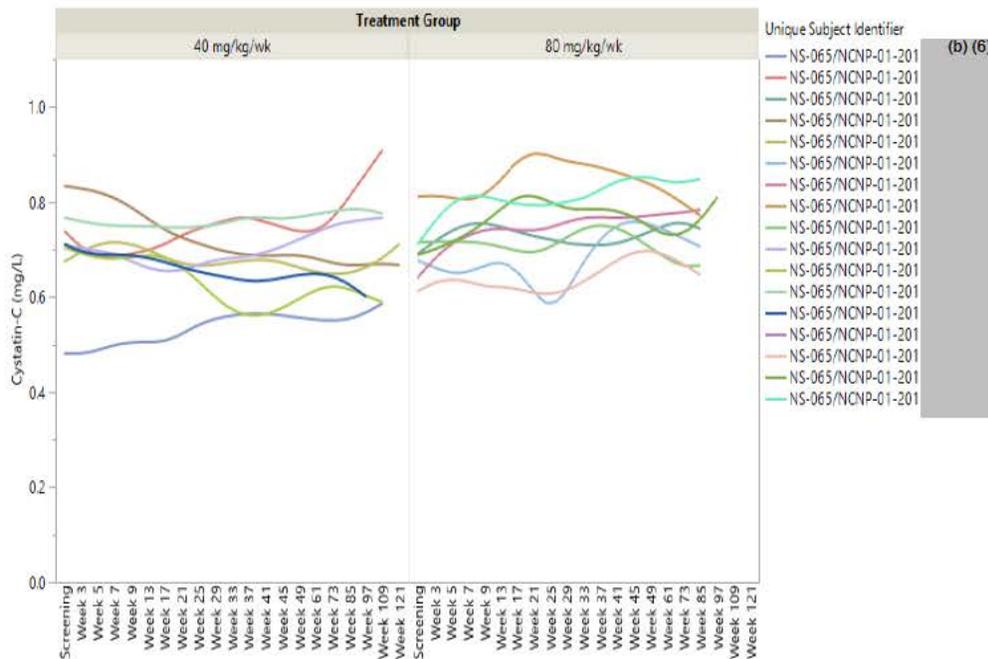
Figure 2: Mean change from baseline in serum cystatin C over time in Study 201/202 (top) and Study P1/2 (bottom)



Source: Primary reviewer's analyses.



**Figure 3: Individual patient cystatin C values over time by treatment arm in Study 201/202**



Source: Primary reviewer’s analyses.

*Analyses of Urinary Biomarkers:*

Several urinary biomarkers of renal tubular toxicity were measured in studies 201/202 and P1/P2 (protein, albumin, NAG,  $\alpha$ 1-microglobulin,  $\beta$ 2-microglobulin). Both the applicant and primary review team report that there were no consistent patterns or increases across biomarkers.

The applicant identified seven patients with a shift in NAG level from normal to high (defined as over 11.5 U/L; baseline values ranged from 0.35 to 5.3 U/L) during study P1/2, including the three patients with related AEs noted above (see Appendix B). Three of the patients had only one high value during the trial ( (b) (6) ), and one had intermittent elevated values that normalized despite continued treatment ( (b) (6) ). Three patients, all in the 80 mg/kg/week group ( (b) (6) ) had multiple elevated values that persisted through the end of the study. None of these patients had other findings suggestive of renal injury. The primary review team identified two additional patients with elevations in NAG to  $\geq 10$  U/L during Study 201/202, both in the 80 mg/kg/week group (see Appendix B).

*Reviewer’s comment: It is not clear why cutoffs of 11.5 U/L or 10 U/L were selected for the analyses.*

**Renal Safety Findings in Study DMT01**

In Study DMT01 conducted in Japan, adverse events of beta-N-acetyl-D-glucosaminidase increased were reported for 9 of 10 patients, “protein urine present” for 8 of 10 patients, and “albumin urine present” for 7 of 10 patients. Other renal-related AEs included beta 2-



microglobulin increased and cystatin C increased in two patients each and hematuria, blood urine present, and urine protein/creatinine ratio increased in one patient each. All but one “protein urine present” event were assessed as CTCAE Grade 1.

On further investigation of the urine protein findings, the applicant concluded that viltolarsen interferes with the pyrogallol red dye-binding method of 24-hour urine protein measurement and, using an alternative assay (Coomassie brilliant blue), levels were within the normal range. This informed the assays used in later studies in the development program (i.e., Studies 201/202 and P1/2). The applicant observed, however, that this would not explain the other events reported in this study, which seemed out of proportion to the numbers seen in other viltolarsen studies and the fact that lower viltolarsen doses were administered in DMT01 (i.e., the highest dose administered was 20 mg/kg/week).

**Overview of Ongoing Confirmatory trial NS-065/NCNP-01-301**

The applicant is currently conducting Study NS-065/NCNP-01-301, titled “A Phase 3 Randomized, Double-blind, Placebo-controlled, Multi-center Study to Assess the Efficacy and Safety of Viltolarsen in Ambulant Boys with Duchenne Muscular Dystrophy (DMD).” No patients have been enrolled to date. The trial is intended to verify the treatment benefit of viltolarsen in the post-marketing setting. Up to 74 boys 4 to <8 years of age will be randomized 1:1 to viltolarsen 80 mg/kg or placebo for 48-weeks. The primary efficacy endpoint will be based on the Time to Stand Test (TTSTAND) as a measure of strength and function.

[Redacted] (b) (4)  
[Redacted] (exclusions added with amendment dated March 2, 2020).

[Redacted] (b) (4)  
[Redacted] added with amendment dated March 2, 2020 [Redacted] (b) (4)

[Redacted] (b) (4) (added with amendment dated March 2, 2020):

[Redacted] (b) (4)

[Redacted] (b) (4)

### Consult Questions

**Question 1:** Please comment on the safety of viltolarsen with respect to renal toxicity observed in the viltolarsen development program both from the perspective of TEAEs and Laboratory parameters related to renal function, including biomarkers of renal toxicity, and provide labeling recommendations regarding renal toxicity with viltolarsen and need for monitoring.

*DCN Response: A total of 32 patients have been exposed to viltolarsen for a mean of 12 months in the pooled phase 2 studies. Non-clinical studies of viltolarsen showed evidence of renal tubular toxicity. Renal toxicity, including elevations in serum creatinine, proteinuria, acute kidney injury, and acute glomerulonephritis, has also been observed with other ASOs. Given these findings and this experience, the applicant included regular assessments of serum creatinine, serum cystatin C, and urinary biomarkers of renal tubular toxicity in studies 201/202 and P1/P2. There is no obvious signal for renal toxicity based on either laboratory data or renal-related adverse events (see response to Question 2), though the safety database is limited in size and duration.*

*As we understand, the Division is considering including a Warning and Precaution for renal toxicity based on the preclinical findings and experience with other ASOs, which is consistent with the approach taken with golodirsen in 2019. Given limitations in the available clinical safety database, we believe it would be reasonable to include a Warning and Precaution for renal toxicity with supportive information on the relevant preclinical findings in Section 13.*

**Question 2:** Please comment on the utility of Urine Beta-N-acetyl-D-glucosaminidase levels to assess tubular damage in DMD patients.

*DCN Response: Beta-N-acetyl-D-glucosaminidase (NAG) is an enzyme expressed in the proximal tubules of the kidney and is one of several exploratory urinary biomarkers of kidney injury. Urinary NAG and other urinary biomarkers have been qualified by the FDA as part of a safety biomarker panel to aid in the detection of kidney tubular injury in phase I trials in healthy volunteers (<https://www.fda.gov/drugs/cder-biomarker-qualification-program/reviews-qualification-biomarker-clusterin-clu-cystatin-c-cy5c-kidney-injury-molecule-1-kim-1-n>). Excretion of NAG into the urine correlates with proximal tubular cell injury and, in studies of various renal diseases including acute kidney injury and treatment with nephrotoxic compounds, increased urinary NAG levels have typically been observed before increases in serum creatinine.*

*In the viltolarsen studies, intermittent elevations in urinary NAG above a specified threshold were noted in some patients without other findings suggestive of renal toxicity. The clinical significance of the observed changes and the utility of NAG in detecting tubular damage, both in this population and in general, is unclear.*

*Additional comment:*

*Given the aforementioned findings for urinary NAG, you may want to consider looking at the distribution of the change from baseline in NAG to (1) the end of the placebo-controlled period (Study 201/202) and (2) the end of treatment (Studies 201/202 and P1/P2) by treatment arm (i.e.,*



*a figure that shows number of subjects on the y-axis and change from baseline in NAG on the X-axis). Such analyses may give you a better sense of whether the drug is altering urinary NAG levels.*

**Question 3:** Please advise on the adequacy of the renal monitoring in the protocol for confirmatory study NS-065/NCNP-01-301, a 48-week confirmatory study in exon 53 skippable DMD patients.

*DCN Response: The confirmatory study, which has not enrolled any patients to date, will randomize up to 74 boys 4 to <8 years of age 1:1 to viltolarsen 80 mg/kg or placebo for 48-weeks.* (b) (4)

*As previously noted, there has been no obvious signal for renal toxicity in the clinical development program to date, though the safety database is very limited. We believe the proposed confirmatory trial provides an important opportunity better characterize this potential risk and have the following comments and recommendation related to optimizing the design of the trial to do so.* (b) (4)



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## References

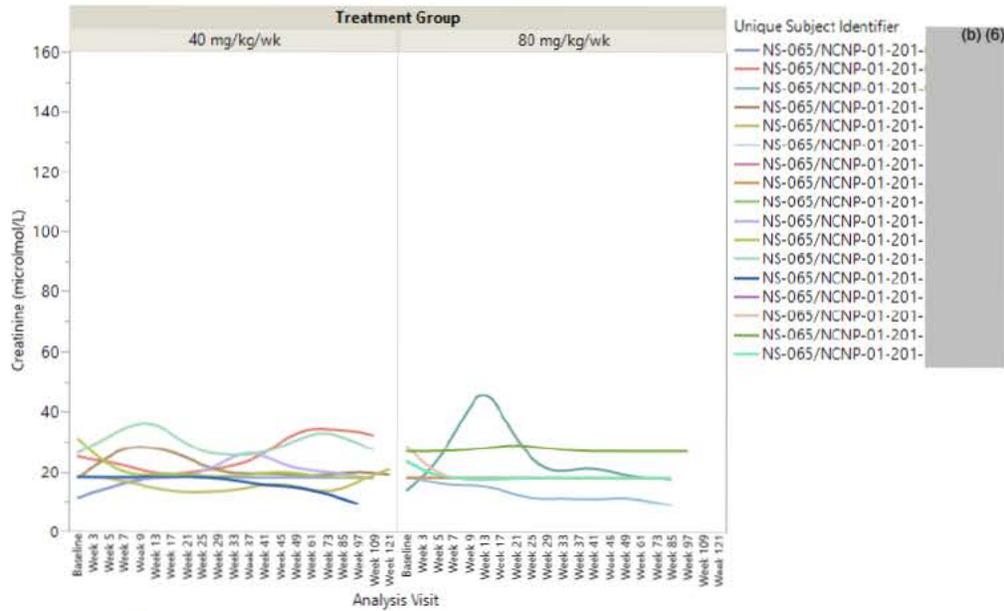
- Engelhardt, J. A. "Comparative Renal Toxicopathology of Antisense Oligonucleotides." *Nucleic Acid Ther* 26, no. 4 (Aug 2016): 199-209. <https://doi.org/10.1089/nat.2015.0598>.  
<https://www.ncbi.nlm.nih.gov/pubmed/26983026>.
- Price, R. G. "Measurement of N-Acetyl-Beta-Glucosaminidase and Its Isoenzymes in Urine Methods and Clinical Applications." *Eur J Clin Chem Clin Biochem* 30, no. 10 (Oct 1992): 693-705. <https://www.ncbi.nlm.nih.gov/pubmed/1493161>.
- van Poelgeest, E. P., R. M. Swart, M. G. Betjes, M. Moerland, J. J. Weening, Y. Tessier, M. R. Hodges, A. A. Levin, and J. Burggraaf. "Acute Kidney Injury During Therapy with an Antisense Oligonucleotide Directed against Pcsk9." *Am J Kidney Dis* 62, no. 4 (Oct 2013): 796-800. <https://doi.org/10.1053/j.ajkd.2013.02.359>.  
<https://www.ncbi.nlm.nih.gov/pubmed/23561896>.



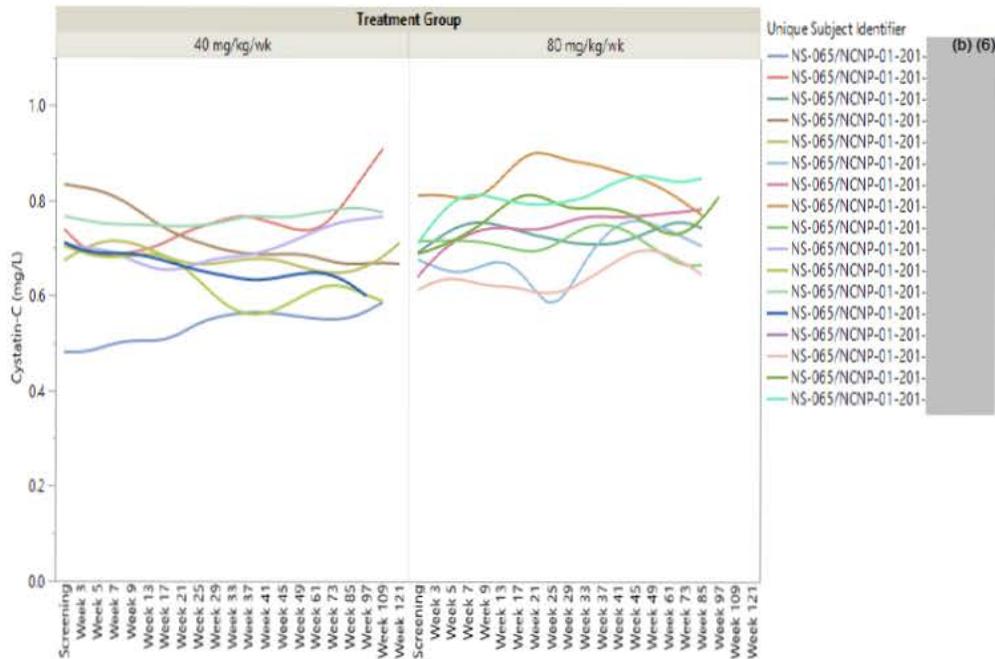
Appendix A: Individual patient creatinine and cystatin C data by study.

Study 201/202

Creatinine

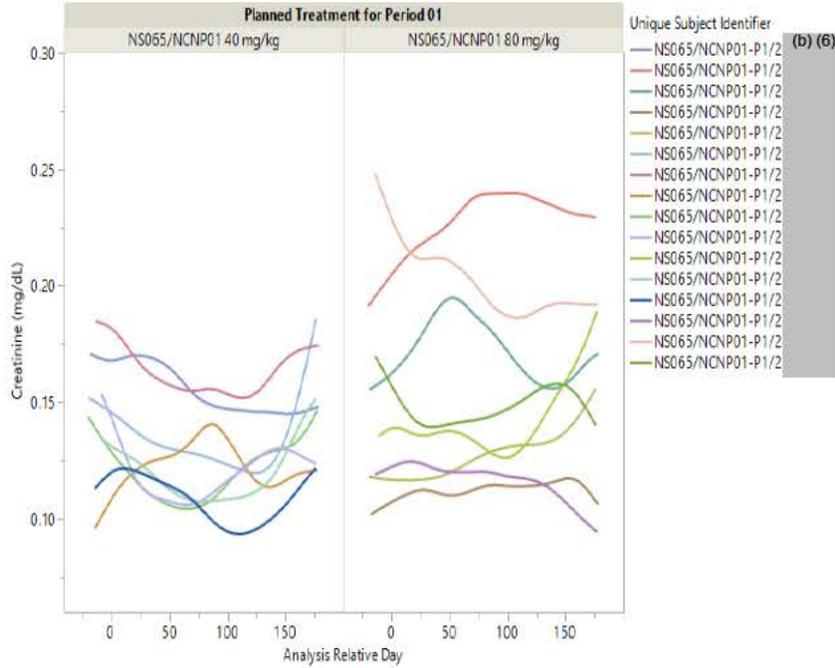


Cystatin C

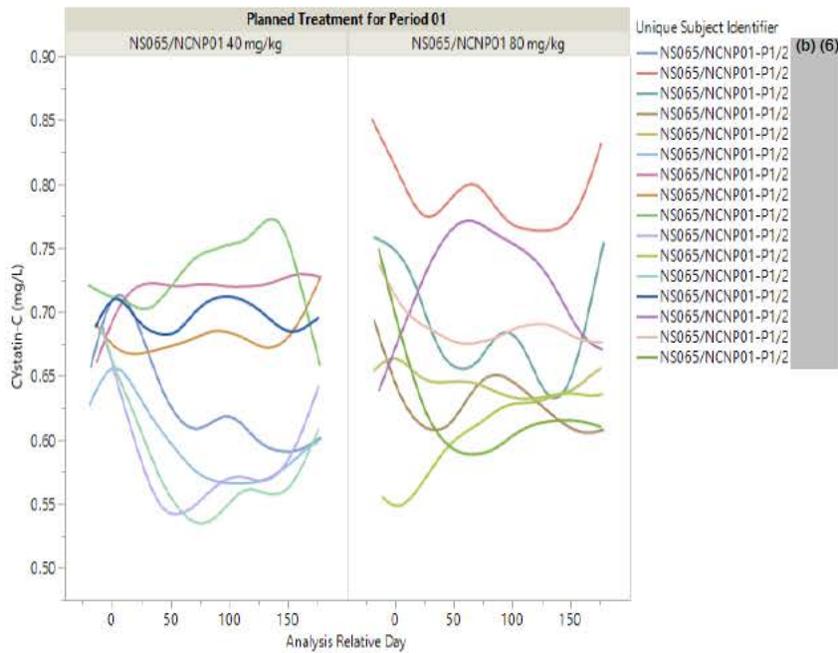




### Study P1/2 Creatinine

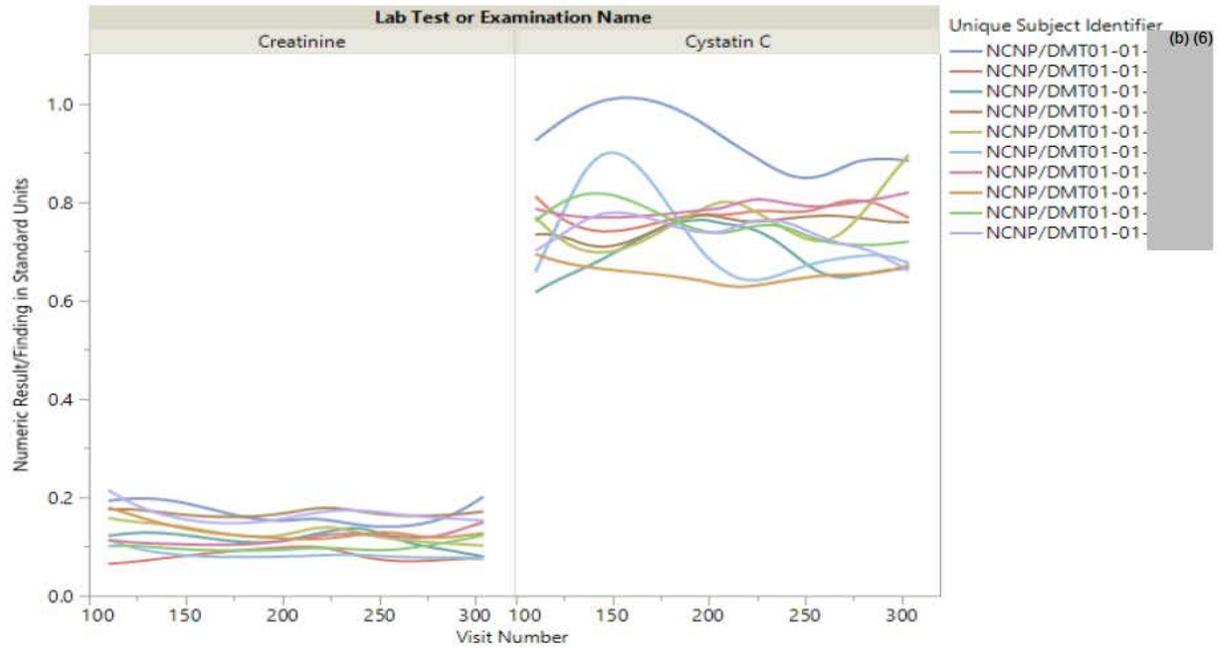


### Cystatin C





**Study DMCT01**





**Appendix B: Patients with NAG levels exceeding specified thresholds**

**Patients with elevations in NAG levels from baseline to  $\geq 11.5$  U/L in Study P1/2**

Patient	Dose	Week	Value (U/L)	
(b) (6)	40	18	21.2	
	40	19	12.1	
	40	6	6	13.2
			12	11.8
			13	16.6
			14	12.1
	80	15	12.3	
	80	20	20	16.7
			21	16.1
			23	19.6
			25	15.1
	80	17	17	13.6
			20	19.9
			24	13.0
	80	8	8	12.3
			14	16.5
20			14.7	
24			13.5	

Source: Applicant, Summary of Clinical Safety, Table 40.

**Patients with elevations in NAG levels from baseline to  $\geq 10$  U/L in Study 201/202**

Study 201/202 Patient	Age (Years)	Viltolarsen Dose	Week(s) of High Observation(s)	Post-baseline High NAG Value (U/L)
(b) (6)	(b) (6)	80 mg/kg/wk	17	12.76
			21	11.72
			29	10.73
			41	12.8
			49	10.35
(b) (6)	(b) (6)	80 mg/kg/wk	33	13.49
			41	10.02

Source: Primary reviewer's analyses.

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