

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

212154Orig1s000

NON-CLINICAL REVIEW(S)

MEMORANDUM

**DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research**

Date: August 10, 2020

From: Lois M. Freed, Ph.D.
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Acting Director, Division of Pharmacology/Toxicology-Neuroscience
Office of Neuroscience

Subject: NDA 212-154 (Viltepso, viltolarsen, NS-065/NCNP-01)

The NDA to support viltolarsen for the treatment of Duchenne Muscular Dystrophy in patients with exon deletions amenable to skipping of exon 53 was submitted by Nippon Shinyaku Co. Ltd. in two parts. The nonclinical data were received on February 1, 2019; the clinical and CMC data were received on September 30, 2019. The NDA was filed (Filing Communication, February 6, 2020), with no filing review issues identified. Clinical development of viltolarsen was conducted under IND 127474.

The nonclinical studies conducted to support clinical development and marketing authorization for viltolarsen included the following: pharmacology, PK/ADME, 26- and 39-week toxicity studies in male mouse and monkey, respectively, fertility and early embryonic development in male mouse, and juvenile animal toxicology studies in male mouse. The nonclinical data were reviewed by Dr. David Carbone (Pharmacology/Toxicology NDA Review and Evaluation, July 16, 2020), who has concluded that the application is approvable from a nonclinical standpoint. Dr. Carbone notes that carcinogenicity studies of viltolarsen are to be conducted as post-marketing requirements (PMRs).

Selected nonclinical data are discussed below; a comprehensive description and discussion of the nonclinical studies are provided in Dr. Carbone's review.

Pharmacology

Viltolarsen is a 21-mer phosphorodiamidate morpholino (PMO) antisense oligonucleotide (ASO) designed to bind to exon 53 of dystrophin pre-mRNA, resulting in exclusion of this exon during mRNA processing in patients with

genetic mutations that are amenable to exon 53 skipping and production of a truncated but functional dystrophin protein.

Primary: In vitro proof-of-concept studies, conducted primarily in DMD patient fibroblasts differentiated into myotubes, demonstrated viltolarsen-induced exon 53 skipping (EC₅₀ values of 0.63-0.90 µM and 2.3 µM) and production of dystrophin protein in cells from DMD patients with deletion of exons 45-52 and exons 48-52, respectively.

In vivo assessment of exon 53 skipping was included in the 12- and 39-week toxicity studies in monkey; viltolarsen is not pharmacologically active in rodents.

Secondary: The potential for off-target hybridization was evaluated in silico and in vitro (HEK293 and ITO-II cells) for viltolarsen and metabolites (n+1, n-1, n-2 oligomers). Twenty potential off-target genes were identified in humans; however, only one (*APCCDD1*) was considered to have possible relevance for humans. Mutations at this locus are associated with hereditary hypotrichosis, which the sponsor notes “is occasionally accompanied by light-colored or hypopigmented hair shafts.” “Hair color changes,” reported in one patient, were considered possibly drug-related.

PK/ADME

Serum protein binding was low (≤40%) in all species tested (mouse, rat, monkey, and human), which is characteristic of PMO ASOs.

Tissue distribution studies of radiolabeled viltolarsen were conducted in male monkey. Following an acute 20-mg/kg IV dose, there was extensive distribution of dose radioactivity primarily to the kidney cortex (peak concentration of 461 µg-Eq/g at 24 hrs post dose); peak levels in skeletal muscles were 5.81-7.41 µg-Eq/g at 0.25 hrs post dose). With multiple doses (20 mg/kg/week for 8 weeks), highest levels of dose radioactivity were in kidney cortex (819 µg-Eq/g), with concentrations in skeletal muscle of 0.331- 0.479 µg-Eq/g.

Toxicology

The pivotal IV toxicity studies were conducted in male CD-1 mouse (13 and 26 weeks) and male cynomolgus monkey (12 and 39 weeks).

Mouse: Viltolarsen was administered by IV injection for 13 weeks (+4-week recovery) and 26 weeks (+8-week recovery) at doses of 0, 60, 240, and 1000 mg/kg QW and 0, 15, 60, 240, and 1000 mg/kg QW, respectively. The primary toxicity in both studies was observed in kidney.

In the 13-week study, there were no drug-related deaths; however, in the 26-week study, two high-dose (one main-study and one satellite) males (HDM) died

prematurely (Day 140 and 169). The death in the main-study HDM was attributed to nephrotoxicity; kidney findings in the satellite HDM were stated to be similar to those in the main-study HDM (basophilic material in the distal tubule and/or collecting duct, dilatation of the distal tubules and/or collecting duct, vacuolation of the epithelium of the distal tubule and/or collecting duct, fibrosis).

In both studies, kidney histopathology findings similar to those in the HD deaths were observed in survivors at the HD and, to a lesser extent, at the 240-mg/kg dose, except that fibrosis was only detected in the 26-week study, at the HD.

Clinical pathology findings consistent with nephrotoxicity (increased BUN, creatinine, and cystatin C), as well as increases in C-reactivity protein, were observed at 1000 mg/kg in both studies.

The NOAEL for viltolarsen-induced toxicity is 60 mg/kg/week IV, which, in the 26-week study, was associated with plasma exposures ($AUC_{(0-24\text{ hr})}$) of 67.47 $\mu\text{g}\cdot\text{hr}/\text{mL}$ on Day 175. (Plasma levels were <LLOQ by 4 hrs post dose.)

For comparison, plasma exposure ($AUC_{(0-24\text{ hr})}$) in humans (Study 201) at the recommended dose of 80 mg/kg/week IV is 387375 $\text{ng}\cdot\text{hr}/\text{mL}$ or 387 $\mu\text{g}\cdot\text{hr}/\text{mL}$ ($t_{1/2} = 8\text{ hrs}$).

Monkey: Viltolarsen was administered by IV injection for 12 weeks (+4-week recovery) and 39 weeks (+8-week recovery) at doses of 0, 60, 200, and 600 mg/kg QW and 0, 10, 60, and 360 mg/kg QW, respectively.

As in mouse, the primary drug-related toxicity was observed in kidney. Histopathology findings in kidney (accompanied by increases in BUN only at 600 mg/kg) were similar to those in mouse but of lesser severity; renal fibrosis was not detected, and no deaths occurred.

The NOAEL for viltolarsen-induced toxicity is 60 mg/kg, which, in the 39-week study, was associated with plasma exposures ($AUC_{(0-24\text{ hr})}$) of 261.1 $\mu\text{g}\cdot\text{hr}/\text{mL}$ on Day 259.

Deaths in the 26-week mouse study occurred at plasma exposures ~3 and 8 times those achieved at the high doses in the 12- and 39-week studies, respectively, in monkey.

Reproductive and Developmental Toxicology

Embryofetal development and pre- and postnatal development studies were not conducted, as agreed to by the Division, because of the intended patient population, which is almost exclusively male. A fertility and early embryonic development study was conducted in male CD-1 mice, which were mated with untreated females.

In the fertility study, viltolarsen was administered IV at doses of 0, 60, 240, and 1000 mg/kg/week for 9 weeks prior to and during the mating period. No adverse effects were observed. Plasma exposure ($AUC_{(0-24hr)}$) at the HD was 7283 $\mu\text{g}\cdot\text{hr}/\text{mL}$ on Day 63 of dosing.

Juvenile Animal Toxicology

The potential adverse effects of viltolarsen on postnatal development were assessed in juvenile male CD-1 mouse, with dose selection based on the results of two dose-ranging studies.

In the dose-ranging studies, viltolarsen was administered weekly beginning on postnatal day (PND) 7. In the first study, doses up to 1000 mg/kg were administered by subcutaneous (SC) injection on PNDs 7 and 14 and by IV injection on PNDs 21 and 28. Kidney findings included increases in kidney weight and basophilic granules in the tubular epithelium at all but the low dose of 60 mg/kg; no clinical chemistry correlates were observed. In the second study, doses up to 2000 mg/kg were administered by SC injection on PND 7 and by IV injection on PNDs 14, 21, and 28. Kidney findings included basophilic granules in tubular epithelium at all but the low dose of 60 mg/kg and tubular degeneration and/or necrosis, tubular dilatation, and hyaline casts at 1000 and 2000 mg/kg. BUN was increased at all doses.

In the pivotal study, viltolarsen (0, 15, 60, 240, and 1200 mg/kg) was administered weekly by SC injection on PND 7 and by IV injection on PNDs 14 through 70. Drug-related deaths occurred in 5 HDM (PNDs 24-70), with renal tubular degeneration “that may have contributed to their early death.” Renal tubular degeneration was observed in HD survivors and at 240 mg/kg, but no clinical chemistry correlates were reported. Reduced body weight gain and delayed sexual maturation was observed at the HD. Other developmental parameters were not adversely affected. The NOAEL of 60 mg/kg, based on kidney toxicity, was associated with plasma exposure ($AUC_{(0-24hr)}$) of 542.4 $\mu\text{g}\cdot\text{hr}/\text{mL}$.

Plasma exposures achieved in adult and juvenile mice could not be directly compared because of the decrease in exposures during the dosing period in juvenile animals, possibly due to the smaller number of animals evaluated at the last sampling time (PND 70).

Genetic Toxicology

Viltolarsen was negative in a standard battery of in vitro (Ames, chromosomal aberration in CHL cells) and in vivo (mouse micronucleus) assays.

Carcinogenicity

Carcinogenicity studies of viltolarsen were not submitted to the NDA, as agreed to by the Division. Protocols for a 26-week study in Tg.rasH2 mouse and a 2-year study in rat were submitted for review under Special Protocol Assessment and were reviewed by the Division and the Executive CAC under IND 127474 (Minutes dated July 12, 2018, and August 9, 2019, respectively).

Recommendations

The nonclinical studies of viltolarsen are adequate to support approval of the NDA for the proposed indication. If the application is approved, a 26-week carcinogenicity study in Tg.rasH2 mouse and a 2-year carcinogenicity study in rat should be conducted as PMRs.

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/s/

LOIS M FREED
08/10/2020 09:22:00 AM

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number: 212154
Supporting document: 1, 5
Applicant's letter date: February 1, 2019
CDER stamp date: February 1, 2019
Product: Vitolarsen
Indication: Duchenne Muscular Dystrophy
Applicant: Nippon Shinyaku Co., Ltd.
Review Division: DN1
Reviewer: David L. Carbone, Ph.D.
Supervisor: Lois M. Freed, Ph.D.
Acting Division Director: Eric Bastings, M.D.
Project Manager: Annie Nguyen, R.Ph.

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1 Executive Summary

1.1 Introduction

Vitolarsen has been developed by Nippon Shinyaku Co. Ltd. for the treatment of Duchenne muscular dystrophy.

1.2 Brief Discussion of Nonclinical Findings

Vitolarsen is a morpholino antisense oligonucleotide intended to induce exon 53 skipping in the human dystrophin gene. A dose of 80 mg/kg vitolarsen is to be administered by weekly 1 h IV infusion. There are no safety concerns regarding impurities or excipients.

Sequence analyses of vitolarsen and n+1, n-1, or n-2 oligomers indicated the potential for off-target interaction with 19 genes. In vivo gene expression analysis and a single occurrence of lighter hair color in clinical trials for vitolarsen suggest possible interactions with mRNA for APCDD1, which is involved in hair pigmentation or loss; however, concern over such an effect is minimal given the intended indication (i.e., DMD).

Pivotal general toxicology studies included weekly IV dosing in adult male CD1 mice and cynomolgus monkeys for 26 or 39 weeks, respectively, and a juvenile animal toxicology study in which male CD1 mice were administered vitolarsen by weekly IV injection for 10 weeks, beginning on PND 7. The NOAEL in all three studies was 60 mg/kg based on kidney toxicity characterized by tubule vacuolation and degeneration.

There were no drug effects on fertility or uterine parameters in male CD1 mice administered weekly doses up to 1000 mg/kg vitolarsen by IV injection for 9 weeks prior to mating with control females. Vitolarsen was negative in Ames, in vitro chromosomal aberration, and in vivo mouse micronucleus assays. Assessments of carcinogenicity in TgRasH2 mice and SD rats are to be conducted as a post-marketing requirement.

1.3 Recommendations

1.3.1 Approvability

Vitolarsen is approvable from a nonclinical perspective.

1.3.2 Additional Nonclinical Recommendations

None

1.3.3 Labeling

8.1 Pregnancy

Risk Summary

There are no human or animal data available to assess the use of VILTEPSO during pregnancy. In the U.S. general population, major birth defects occur in 2 to 4% and miscarriage occurs in 15 to 20% of clinically recognized pregnancies.

(b) (4)

8.2 Lactation

Risk Summary

There are no human or animal data to assess the effect of VILTEPSO on milk production, the presence of vitolarsen in milk, or the effects of VILTEPSO on the breastfed infant.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for VILTEPSO and any potential adverse effects on the breastfed infant from VILTEPSO or from the underlying maternal condition.

(b) (4)

8.4 Pediatric Use

VILTEPSO is indicated 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, including pediatric patients [see Clinical Studies (14)].

(b) (4)

12.1 Mechanism of Action

VILTEPSO is designed to bind to exon 53 of dystrophin pre-mRNA resulting in exclusion of this exon during mRNA processing in patients with genetic mutations that are amenable to exon 53 skipping. Exon 53 skipping is intended to allow for production of an internally truncated dystrophin protein in patients with skipping (b) (4)

(b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

Carcinogenicity studies have not been conducted with vitolarsen.

(b) (4)

Mutagenesis

Vitolarsen was negative in in vitro (bacterial reverse mutation and chromosomal aberration in CHL (b) (4) cells) and in vivo (mouse bone marrow micronucleus) assays.

(b) (4)

Impairment of Fertility

(b) (4) intravenous administration of vitolarsen (0, 60, 240, or 1000 mg/kg) to male mice prior to and during mating with untreated female (b) (4) did not (b) (4)

(b) (4)

Plasma exposure (AUC) at the highest doses (b) (4) approximately

(b) (4) times that in humans at the recommended (b) (4) dose of 80 mg/kg.



2 Drug Information

2.1 Drug

CAS Registry Number: N/A

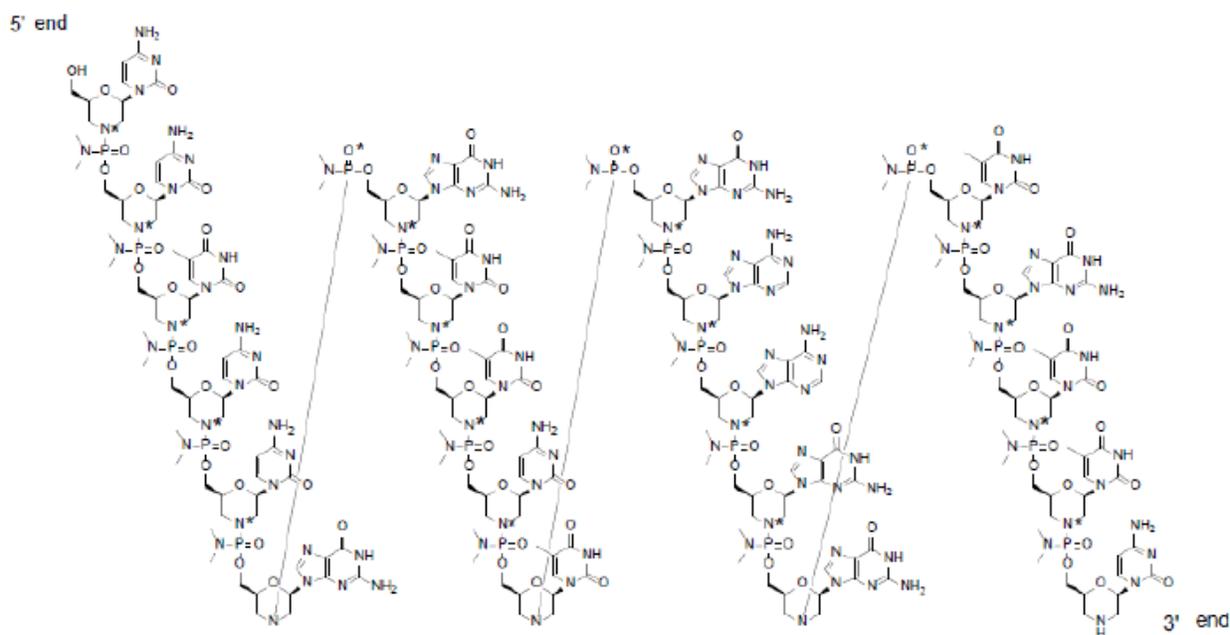
Generic Name: Vitolarsen

Code Name: NS-065/NCNP-01, NS-065, NDK-65

Chemical Name: N/A

Molecular Formula/Molecular Weight: $C_{244}H_{381}N_{113}O_{88}P_{20}$, 6924.82 Da

Structure or Biochemical Description:



Sequence : 5'-CCTCCGGTTCCTGAAGGTGTTTC-3'

(Sponsor's Figure)

Pharmacologic Class: Exon 53-skipping morpholino antisense oligonucleotide

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 127474

2.3 Drug Formulation

Vitolarsen for IV administration is formulated at 50 mg/mL in physiological saline.

2.4 Comments on Novel Excipients

There are no concerns regarding excipients.

2.5 Comments on Impurities/Degradants of Concern

There are no concerns regarding impurities.

2.6 Proposed Clinical Population and Dosing Regimen

Vitolarsen is intended to treat Duchenne muscular dystrophy. The proposed dosing regimen is 80 mg/kg by weekly 1 h IV infusion.

2.7 Regulatory Background

IND 127474

October 20, 2015, pIND

March 25, 2016, IND submission

July 12, 2018, SPA1 (26-week carcinogenicity study in Tg.rasH2 mice)

September 26, 2018, pNDA

August 9, 2019, SPA2 (2-year carcinogenicity study in Wistar rats)

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology

- Exon skipping and tissue localization in *mdx* and *mdx52* mice
- Exon skipping in skeletal and cardiac muscle in cynomolgus monkeys
- In vitro exon skipping and dystrophin production in DMD patient-derived cells
- In silico prediction of off-target interactions for vitolarsen and n+1, n-1, and n-2 oligomers
- Expression of off-target sequences in human-derived cells
- CNS, CV, and respiratory safety pharmacology
- hERG assay

PK/ADME

- Protein binding, blood cell permeability, nuclease stability, metabolic stability
- Distribution and excretion of radiolabeled vitolarsen in rat and monkey

Toxicology

- Single IV or IM dosing in cynomolgus monkey
- 13 and 26-week repeat IV dosing in CD1 mice
- 12-week repeat IV and IM dosing in cynomolgus monkey
- 39-week repeat IV dosing in cynomolgus monkey
- Ames, in vitro chromosomal aberration, and in vivo mouse micronucleus (b) (4) manufacturing methods)
- Ames and in vitro chromosomal aberration (b) (4) manufacturing method)
- 5-week repeat IV dosing in cynomolgus monkey ((b) (4) (b) (4) bridging studies)
- 13-week repeat IV dosing, Ames, and in vitro chromosomal aberration ((b) (4) impurity qualification studies)
- Fertility study in which only male CD1 mice received vitolarsen
- Juvenile animal range-finding and pivotal toxicology studies in male CD1 mice

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

IND 127474

Nonclinical review by C. Toscano, Ph.D. (5/25/2016).

Nonclinical review of SPA1, by C. Toscano, Ph.D. (7/13/2018).

Nonclinical review of SPA2, by C. Toscano, Ph.D. (8/9/2019).

4 Pharmacology

4.1 Primary Pharmacology

Studies with a mouse homolog of vitolarsen (NDK-73) indicated an EC₅₀ of 6.1 μM for exon skipping following IV administration. An additional study in the *mdx52* mouse, which lacks exon 52 of the dystrophin gene, indicated skipping efficiency of up to 4.8% following IV administration of up to 640 mg/kg NDK-73 in combination with 640 mg/kg NDK-33, which targets a different region of exon 53. IV administration of 60 mg/kg vitolarsen resulted in up to 6.0% exon skipping efficiency cynomolgus monkeys. In the 39-week study in male cynomolgus monkeys, exon skipping efficiency was also near 6.0% following weekly IV administration of 360 mg/kg. Studies in rhabdomyosarcoma cells isolated from DMD patients indicated EC₅₀ values ranging from 3.4 to 5.9 nM for exon 53 skipping. In myotubes derived from fibroblasts isolated from DMD patients, transfection with vitolarsen resulted in exon 53 skipping efficiency up to 83.1%, and Western blot analysis indicated the presence of dystrophin.

4.2 Secondary Pharmacology

Sequence analysis of vitolarsen and n+, n-1 or n-2 oligomers indicated a possible off-target interaction with 19 genes (ALDH1A2, APCDD1, CAMKK2, CNTNAP2, FSHR, FUT1, LMTK2, LRIG1, MYT1, PCDH15, PRKCH, RP11-45901.2, RP11-479016.1, SLC22A10, SLC24A2, TIAM1, WDR20, WRN, ZNF557). In vitro gene expression studies in HEK293 and ITO-II cells indicated the potential for drug-mediated changes in mRNA levels for APCDD1, CNTNAP2, FUT1, and MYT1.

4.3 Safety Pharmacology

Summarized from nonclinical review of IND 127474:

CNS safety pharmacology endpoints were assessed by FOB in SD rats administered a single IV dose of up to 500 mg/kg; there were no remarkable findings. Cardiovascular and respiratory endpoints were assessed in cynomolgus monkeys administered a single IV dose of up to 600 or 500 mg/kg, respectively. There were no drug effects on blood pressure, heart rate, or ECG (PR, QRS, QT, and QTc with Bazett's correction), or respiratory rate or oxygen saturation. Additionally, there were no drug-related effects on hERG current in CHO cells exposed to 0, 0.3, 1, or 3 mg/mL NS-065.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Serum levels of vitolarsen in mouse and monkey were quantified using validated ELISA methods.

Intravenous administration of 6, 20, and 60 mg/kg [¹⁴C]-vitolarsen in male SD rats and cynomolgus monkeys resulted in generally linear increases in plasma AUC. Excretion

was not evaluated in rat; however, over 75% of drug-related radioactive material in monkey was excreted in urine over a 24 h period.

Distribution and binding studies in RBCs isolated from rat, monkey, and human indicated minimal (i.e., 2.5 to 3.5%) uptake, and serum protein binding ranging from 29.7 to 40.3%. Administration of 20 mg/kg [¹⁴C] vitolarsen in male *mdx* and WT mice indicated accumulation in all tissues, with the highest amounts in kidney, cardiac whole blood, plasma, and liver, and the lowest concentrations of radioactive material in CNS, eye, seminal vesicles, and bile. 15 minutes after dosing, the maximum concentrations were reached in most tissues, and drug concentrations were still highest in kidney (435 to 457 µg equiv/g); by comparison, drug concentrations in lower forelimb and hindlimb skeletal muscle were 47.3 and 40.7 µg equiv/g, respectively. Single doses of 20 mg/kg [¹⁴C] vitolarsen in male cynomolgus monkeys indicated accumulation in kidney, adrenal gland, cardiac whole blood, thyroid, liver, spleen, and GI tract. Repeat IV doses of 20 mg/kg in male cynomolgus monkey resulted in accumulations of radioactive material in kidney, liver, testis, adrenal gland, and spleen.

6 General Toxicology

6.1 Single-Dose Toxicity

A single IV dose of 0 or 600 mg/kg vitolarsen was administered to male cynomolgus monkeys (1/group), resulting in drug-related vacuolation in the epithelium of the proximal renal tubule.

Single intramuscular doses of 0, 1, 10, or 100 mg/kg vitolarsen were administered to male cynomolgus monkeys. Each group consisted of 8 animals; 5 were necropsied on the day of dosing, and the remaining 3 were necropsied on Day 14. There were no drug-related clinical signs. Drug-related effects included increases in AST and CK, and degeneration and necrosis at the injection site. It was unclear why AST was elevated in controls; however, there were no remarkable effects on clinical chemistry parameters in recovery animals.

Finding	Day 1 (mg/kg)				Day 14 (mg/kg)			
	0	1	10	100	0	1	10	100
AST (U/L)	128	156	171	266	43	40	42	34
CK (U/L)	1968	2183	4600	130004	495	301	172	233
<i>Injection Site Histology</i>								
Degeneration/Necrosis	4/5	1/5	5/5	3/5	N/A	N/A	N/A	N/A
very slight	3/5	1/5	0	0	N/A	N/A	N/A	N/A
slight	1/5	0	3/5	1/5	N/A	N/A	N/A	N/A
moderate	0	0	2/5	2/5	N/A	N/A	N/A	N/A
Regeneration	N/A	N/A	N/A	N/A	0/3	0/3	3/3	2/3

6.2 Repeat-Dose Toxicity

MOUSE

(13-week, repeat IV administration; summarized from nonclinical review of IND 127474:

Male CD1 mice (15/group) received a weekly IV infusion of 0, 60, 240, or 1000 mg/kg vitolarsen for 13 weeks, followed by a 4-week recovery period. There were no drug-related clinical signs. Drug effects on hematology parameters included decreases in hematocrit and MCH in HDM relative to control; effects on clinical chemistry parameters included increases in urea nitrogen (2.5x), creatinine (1.4x), cystatin C (1.6x), and CRP (1.6x) in HDM relative to controls. Changes in hematocrit, MCH, and CRP resolved over the recovery period, but urea nitrogen, creatinine, and cystatin C remained elevated. Mean absolute kidney weight was increased in MDM (687 mg) and HDM (705 mg) relative to controls (611 mg) at the end of the dosing phase, and in HDM (699 mg) relative to control (626 mg) after the recovery period. Histology findings after dosing and recovery periods included intratubular basophilic material, dilation, and epithelial vacuolation in the distal tubule and collecting duct in MDM and HDM.

Kidney Finding	Left Kidney (mg/kg)				Right Kidney (mg/kg)			
	0	60	240	1000	0	60	240	1000
Main								
Intratubular basophilic material	0/15	0/15	5/15	6/15	0/15	0/15	3/15	8/15
Distal tubule dilation	0/15	0/15	1/15	14/15	0/15	0/15	1/15	14/15
Proximal tubule vacuolation	0/15	1/15	0/15	1/15	0/15	0/15	0/15	0/15
Distal tubule vacuolation	0/15	0/15	0/15	0/15	0/15	0/15	13/15	15/15
Recovery								
Intratubular basophilic material	0/15	0/15	4/15	9/15	0/15	0/15	7/15	9/15
Distal tubule dilation	0/15	0/15	2/15	12/15	0/15	0/15	2/15	12/15
Distal tubule vacuolation	0/15	0/15	9/15	15/15	0/15	0/15	10/15	15/15

TK parameters were evaluated on Days 0 and 84 in satellite animals. Increases in C_{max} and AUC were greater than dose proportional. Based on renal toxicity, the NOAEL was 60 mg/kg.

Toxicokinetics parameters for NS-065 are shown in the following table:

Group	Dose Level (mg/kg)	Sampling Point (Day of dosing)	C_{max} ($\mu\text{g/mL}$)	T_{max} (h)	AUC _{0-24h} ($\mu\text{g}\cdot\text{h/mL}$)
10	60	0	187.8	0.1	57.93
		84	230.0	0.1	83.48
11	240	0	749.3	0.1	582.2
		84	854.4	0.1	420.6
12	1000	0	4580	0.1	7946
		84	5535	0.1	12300

(Sponsor's Table)

Study title: A 26-week Intermittent Intravenous Dose Toxicity Study of NS-065 (NDK-65) in Mice Followed by an 8-week Recovery Period

Study no.: TX10827
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)

Date of study initiation: April 1, 2015
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: NDK-65, Lot 57, 97.1%

Methods

Doses: 0, 15, 60, 240, 1000 mg/kg
 Frequency of dosing: Weekly
 Route of administration: IV
 Dose volume: 10 mL/kg
 Formulation/Vehicle: Saline
 Species/Strain: CD1 Mice
 Number/Sex/Group: 15 males/group (main and recovery)
 Age: 7 weeks at initiation of dosing
 Weight: 29.4 to 38.3 g
 Satellite groups: TK (33/group); serum cytokine and complement analysis (35/group); ADA (5 to 10/group)
 Unique study design: Male-only; recovery was not evaluated at 15 mg/kg.
 Deviation from study protocol: None

Observations and Results**Mortality and Clinical Signs**

Animals were observed 1 to 2 times daily for mortality or signs of morbidity. HDM Nos. 129 (main study) and 566 (cytokine/complement satellite group) died on Days 140 and 169, respectively. COD in these animals was thought to be due to kidney toxicity, based on findings of grossly enlarged kidneys, epithelial vacuolation in the proximal tubules, distal tubules, and collecting ducts, and dilation of the distal tubules.

Body Weights and Food Consumption

Body weights and food consumption were evaluated weekly. Mean body weights were decreased by up to 6 g from Weeks 12 through 25 in HDM relative to controls. Body

weight gain in HDM increased during the recovery period, but mean body weight did not reach that of controls. There was no drug effect on food consumption.

Ophthalmoscopy

Observations by slit lamp and indirect ophthalmoscopy were conducted prior to the initiation of dosing, on Days 85 and 169 of the dosing period and on Day 49 of the recovery period. There were no drug-related findings.

ECG

Not evaluated.

Hematology, Clinical Chemistry, and Urinalysis

Blood samples were collected at scheduled necropsy. Urine samples were collected during Weeks 13 and 25 of the dosing period and on Days 51 or 52 of the recovery period. Hematology findings included decreases in hemoglobin, hematocrit, MCV, and MCH in MDM; after the recovery period, decreases in erythrocyte count, hemoglobin concentrations, and hematocrit were observed in HDM. Drug effects on clinical chemistry parameters, including increases in AST, urea nitrogen, creatinine, and cystatin C, were observed in HDM. After the recovery period, urea nitrogen, creatinine, and cystatin C were elevated in HDM. Urine pH was decreased in HDM (pH = 5 to 6) (pH = 7 to 8.5) during the dosing and recovery periods.

inding	Dosing (mg/kg)				
	0	15	60	240	1000
<i>Main</i>					
RBC (10 ⁶ /uL)	7.829	8.136	8.406	7.710	7.500
Hb (g/dL)	11.76	12.40	12.29	11.65	10.68*
HCT (%)	36.44	38.26	37.84	36.19	33.23*
MCV (fL)	46.73	47.09	45.09	46.96	44.34*
MCH (pg)	15.04	15.25	14.61	15.13	14.23*
AST (U/L)	46.3	58.9	48.1	53.4	65.5*
Urea nitrogen (mg/dL)	21.29	21.9	21.83	22.61	48.20*
Creatinine (U/L)	0.096	0.089	0.086	0.094	0.127*
Cystatin C (ng/mL)	706.811	618.287	600.201	654.183	1002.002
C-reactive protein (ng/mL)	6158.326	4684.449	4103.179	5308.309	8108.255
<i>Recovery</i>					
RBC (10 ⁶ /uL)	8.078	N/A	8.126	8.001	7.358*
Hb (g/dL)	12.24	N/A	12.21	12.05	10.96*
HCT (%)	37.69	N/A	37.84	36.88	33.24*
MCV (fL)	46.78	N/A	46.58	46.13	45.25
MCH (pg)	15.21	N/A	15.04	15.05	14.94
AST (U/L)	57.6	N/A	56.7	51	55.7
Urea nitrogen (mg/dL)	19.69	N/A	20.04	21.59	44.73*
Creatinine (U/L)	0.080	N/A	0.083	0.096*	0.126*
Cystatin C (ng/mL)	680.496	N/A	624.371	661.084	989.947*
C-reactive protein (ng/mL)	4302.504	N/A	3966.736	5247.714	4308.309

* Statistical difference ($p < 0.05$) from control

Gross Pathology and Organ Weights

Drug-related gross findings included bilateral kidney enlargement in HDM No. 169. There were no drug-related gross findings in animals that survived until scheduled necropsy. Absolute and relative kidney weights were increased HMDM and HDM. Absolute and relative kidney weights were elevated in HDM after the recovery period.

Tissue	Dosing Period (mg/kg)					Recovery (mg/kg)			
	0	15	60	240	1000	0	60	240	1000
Kidney-R ^a	325.3	318.8	337.9	334.7	379.6 ^c	354.5	365.3	356.9	414.1 ^c
Kidney-L ^a	314.9	317.8	331.6	340	371.8 ^c	348.5	350.9	352.1	408.9 ^c
Kidney-R&L ^a	640.3	636.6	669.5	674.7	751.4 ^c	703	716.1	709.1	823.0 ^c
Kidney-R ^b	668.5	661.2	721.8	712.9	907.3 ^c	707.3	706.9	726.3	900.9 ^c
Kidney-L ^b	645.5	659.6	709.3	723.9 ^c	887.9 ^c	692.5	679.2	717	892.7 ^c
Kidney-R&L ^b	1313.8	1320.8	1431.1	1437.1	1795.4 ^c	1399.9	1386.1	1443.2	1793.6 ^c

^a Absolute tissue weight (mg)

^b Relative to body weight

^c Statistical difference ($p < 0.05$) from control

Histopathology

Adequate Battery: Yes

Trachea	Liver	Spleen
Lungs	Gallbladder	Thymus
Tongue	Aorta	Pituitary
Submandibular glands	Heart	Thyroid
Sublingual glands	Kidneys	Parathyroid glands
Esophagus	Urinary bladder	Adrenals
Stomach	Testes	Eyes (with optic nerves)
Duodenum	Epididymides	Lacrimal glands
Jejunum	Prostate	Harderian glands
Ileum	Seminal vesicles	Skeletal muscle
Peyer's patches	Brain	Skin
Cecum	Spinal cord	Diaphragm
Colon	Sciatic nerves	Gross lesions
Rectum	Bone and marrow (sternum)	
Pancreas	Mesenteric LN	

Signed Pathology Report: Yes

Peer Review: No

Histological Findings:

Unscheduled Deaths: Findings at necropsy included epithelial vacuolation, dilation, and intratubular basophilic material in the renal distal tubules and/or collecting ducts in Animals 129 and 566, and epithelial vacuolation in the renal proximal tubules in Animal 129.

Scheduled Necropsy: Drug-related effects at the end of the dosing period were found in kidney, testis, and bladder.

Findings	Dosing (mg/kg)					Recovery (mg/kg)			
	0	15	60	240	1000	0	60	240	1000
Kidney	n=15				n=14	n=15			
Hyalin Cast (R/L)	4/4	0/1	0/2	1/0	4/2	0/0	2/3	3/4	1/2
Cellular Infiltration (R/L)	5/1	0/0	0/2	0/0	7/6	0/0	2/4	1/0	6/6
Fibrosis (R/L)	0/0	0/0	0/0	0/0	3/6	0/0	0/0	0/0	3/2
Distal Tubule/Collecting Duct:									
Tubule basophilia (R/L)	1/3	2/1	0/0	2/1	3/2	1/2	1/2	1/3	6/6
Intratubular basophilic material (R/L)	0/0	0/0	0/0	9/13	14/14	0/0	0/0	8/9	14/14
Dilation (R/L)	0/0	0/0	0/0	1/1	14/14	0/0	0/0	0/1	14/13
Epithelial Vacuolation (R/L)	0/0	0/0	0/0	14/14	14/14	0/0	0/0	14/13	15/15
Proximal Tubule:									
Epithelial Vacuolation (R/L)	0/0	0/0	0/0	8/10	13/14	0/0	0/0	0/0	0/0
Epith. Macrovesicular Vacuolation (R/L)	6/7	2/2	3/3	1/1	7/6	6/6	6/6	1/1	0/0
Testis	n=15				n=14	n=15			
Basophilic Material (R/L)	0/0	0/0	0/0	0/0	10/11	0/0	0/0	0/0	9/10
Urinary Bladder	n=15				n=14	n=15			
Transitional Cell Epithelium									
Eosinophilic material	0	0	3	9	11	0	1	2	8
Large vesicle, intracytoplasmic	0	N/A	N/A	N/A	11	N/A	N/A	N/A	N/A

Special Evaluation

Localization of NDK-65 in Urinary Bladder (Study TX-1774)

In situ hybridization (ISH) was used to evaluate drug concentration in urinary bladders from 3 LDM, indicating colocalization of ISH signal with granular matter identified by H&E staining in the umbrella cells (i.e., outer layer) of the transitional epithelium.

Immunotyping, Serum Cytokine Analysis, Complement Activation

There were no drug effects on immunophenotyping parameters (CD3e⁺, CD3e⁺CD4⁺, CD3e⁺CD8a⁺, CD3⁻CD45R/B220⁺, and CD3⁻CD49b/Pan NK cell⁺). There were no drug effects on IL-1 β , IL-2, IL-4, IL-5, IL-12p70, or IFN- γ . There were no drug effects on C3 after dosing on Day 0; however, on Day 175, there was an approximate 2-fold elevation in serum C3 24 h after dosing in HDM. There were no elevations in C3 after the recovery period.

Toxicokinetics

Increases in C_{max} and AUC were dose-proportional. Anti-drug antibodies were not detected.

Group	Dose Level (mg/kg)	Sampling Point (Day of dosing)	C _{max} (µg/mL)	T _{max} (h)	AUC _{0-24h} (µg·h/mL)
12	15	0	57.70	0.1	20.34
		175	47.11	0.1	17.64
13	60	0	229.7	0.1	75.84
		175	210.2	0.1	67.47
14	240	0	981.1	0.1	654.9
		175	682.4	0.1	500.6
15	1000	0	4660	0.1	7458
		175	4352	0.1	17280

(Sponsor's Table)

Dosing Solution Analysis

Dosing solutions ranged from 98.9 to 108.6% of their respective target concentrations.

MONKEY

(12 week repeat IV or IM administration; summarized from nonclinical review of IND 127474)

Male cynomolgus monkeys (5/group) were administered weekly IV infusions of 0, 60, 200, or 600 mg/kg vitolarsen for 12 weeks, followed by a 4-week recovery period. There were no drug-related clinical signs or effects on ophthalmology, ECG, respiratory, or urinalysis parameters. Effects on hematology and clinical chemistry parameters included decreases in RBC (-15%), hemoglobin (-14%), and hematocrit (-12%), increases in reticulocytes (2.6x), bilirubin (2.9x) urea nitrogen (37%), and C-reactive protein (to 5x) in HDM relative to controls; similar findings were not observed after the recovery period. A 35% increase in mean absolute kidney weight was observed after the dosing period in HDM relative to control but was not seen after the recovery period. Histology findings after the dosing period included epithelial vacuolation and basophilia in the proximal tubules, and edema in the interstitium of the renal medullary ray in HDM; findings after the recovery period included epithelial vacuolation in the proximal renal tubules in HDM.

Kidney Findings	Main (mg/kg)				Recovery (mg/kg)			
	0	60	200	600	0	60	200	600
Basophiliia (prox tubule)	0/3	0/3	1/3	3/3	0/2	0/2	0/2	0/2
Edema (medullary ray)	0/3	0/3	0/3	2/3	0/2	0/2	0/2	0/2
Epithelial vacuolation (prox tubule)	0/3	0/3	1/3	3/3	0/2	0/2	0/2	2/2

TK parameters were evaluated after dosing on Weeks 1 and 12. Increases in C_{max} and AUC were greater than dose proportional. Based on renal toxicity, the NOAEL was 60 mg/kg.

Parameter	Week 1 (mg/kg)			Week 12 (mg/kg)		
	60	200	600	60	200	600
C _{max} (µg/mL)	699.2	2270	7835	744.8	2368	7247
AUC _{0-24h} (µg*h/mL)	276.3	917.1	5842	270.1	963.3	5581

A 12-week study was conducted in which 0 (n=2) or 100 (n=3 main, 2 recovery) mg/kg vitolarsen was administered by weekly IM injection. There were no drug-related clinical signs or effects on body weight, food consumption, or ophthalmoscopy and ECG parameters. Increases in AST (2x), CK-MM (10x), and CRP (4x) were increased, and correlated with histology findings of moderate skeletal muscle degeneration at the injection site; slight muscle degeneration at the injection site was also observed after the recovery period, with no abnormal changes in clinical chemistry parameters. There were no signs of renal toxicity.

Study title: A 39-week Intermittent Dose Toxicity Study of NS-065 (NDK-65) in Cynomolgus Monkeys Followed by an 8-week Recovery Period

Study no.: TX10818
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)
 Date of study initiation: December 10, 2014
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: NS-065, Lot 56, 98.3%

Methods

Doses: 0, 10, 60, 360 mg/kg
 Frequency of dosing: Weekly
 Route of administration: IV infusion
 Dose volume: 10 mL/kg
 Formulation/Vehicle: Saline
 Species/Strain: Cynomolgus monkey
 Number/Sex/Group: 5 (main), 3 (recovery)
 Age: 1 year
 Weight: 1.51 to 2.43 kg
 Satellite groups: None
 Unique study design: Male only; no LDM recovery group.
 Deviation from study protocol: None

Observations and Results

Mortality and Clinical Signs

Animals were monitored 1 or 2 times daily for mortality or signs of morbidity. All animals survived until scheduled necropsy. There were no drug-related clinical signs.

Body Weights and Food Consumption

Body weight was evaluated weekly. Food consumption was evaluated daily. There were no drug effects on body weight or food consumption.

Ophthalmoscopy

Slit lamp and indirect ophthalmoscopy were conducted prior to the initiation of dosing, on study Days 78, 169, and 260 (one day after dosing), and on Day 49 of the recovery period; there were no drug effects.

ECG

ECG recordings were conducted prior to the initiation of dosing, after dosing on Days 77, 168, and 259, and on Day 48 of the recovery period; there were no drug-related findings.

Hematology, Clinical Chemistry, and Urinalysis

Blood samples were collected prior to the initiation of dosing, on Days 71, 162, and 267 (one day postdose), and on recovery Day 55. Urine samples were collected prior to the initiation of dosing, on Days 70, 161, and 266, and on Day 54 of the recovery period. There were no drug effects on hematology, clinical chemistry, or urinalysis parameters.

Gross Pathology and Organ Weights

There were no drug-related gross findings or effects on organ weights.

Histopathology

Adequate Battery: Yes

Trachea	Liver	Mesenteric LN
Lungs	Gallbladder	Submandibular LN
Tongue	Aorta	Spleen
Submandibular glands	Heart	Thymus
Esophagus	Kidneys	Pituitary
Stomach	Urinary bladder	Thyroids
Duodenum	Testes	Parathyroids
Jejunum	Epididymides	Adrenals
Ileum	Prostate	Eyes (with optic nerves)
Peyer's patches	Seminal vesicles	Lacrimal glands
Cecum	Brain	Skeletal muscle
Colon	Spinal cord	Skin
Rectum	Sciatic nerves	Injection site
Pancreas	Bone and marrow (sternum)	

Signed Pathology Report: Yes

Peer Review: No

Histological Findings: Drug-related findings were observed in the kidney. A deposition of basophilic granules was observed in the tubular epithelia in 4/5 LDM, 5/5 MDM, and 4/5 HDM. Additional findings in HDM included epithelial vacuolation in the convoluted proximal tubules (2/5), epithelial vacuolation in the straight proximal tubules (4/5), and dilation of the renal tubules (2/5). Electron microscopy indicated intracytoplasmic and membrane-bound vacuoles in the epithelia of the proximal tubules (3/5) and the collecting duct (1/5). Findings in HDM after the recovery period included deposition of basophilic granules in the tubular epithelia (5/5), focal dilation of the renal tubules (2/5), and vacuolation of the straight proximal tubules (1/5).

Special Evaluation

Serum complement and cytokine analysis

Serum complement activation (CH50 and C3) and cytokine levels (IL-2, IL-4, IL-5, IL-6, TNF, IFN- γ , IL-1 β , IL-1ra, and IL-8) were evaluated on Days 0, 84, 175, and 252 of the dosing period, and on Day 55 of the recovery period; there were no drug effects.

Immunophenotyping

CD3+, CD3+CD4+, CD3+CD8+, CD3-CD20+, and CD3-CD16+ cell populations were evaluated prior to the initiation of dosing, on Days 71, 162, and 267 of the dosing period, and on Day 55 of the recovery period; there were no drug-effects.

Toxicokinetics

No anti-drug antibodies were detected. Increases in C_{max} and AUC were generally dose-proportional. Vitrolarsen concentrations were measured in kidney tissue.

Dose (mg/kg)	10				60				360			
Study Week	1	13	26	38	1	13	26	38	1	13	26	38
C_{max} ($\mu\text{g/mL}$)	137.6	134.6	115.1	122.8	805.9	700.3	598.6	613.9	3665	3522	4198	3711
AUC _{0-24h} ($\mu\text{g}\cdot\text{h/mL}$)	50.25	59.77	43.00	54.69	269.3	274.4	251.9	261.1	2452	2081	2501	2294

Dose (mg/kg)	Animal number	Kidney cortex concentration (µg/g)	Mean ± SD	Kidney medullary concentration* (µg/g)	Mean ± SD
10	9	(b) (4)	420±174	(b) (4)	160±156
	10				
	11				
	12				
	13				
60	17		1380±370		547±446
	18				
	19				
	20				
	21				
360	25		6380±3200		4060±2400
	26				
	27				
	28				
	29				

(Sponsor's Table; drug concentrations in kidney cortex and medulla)

7 Genetic Toxicology

Lots 23 and 57 (Summarized from nonclinical review by C. Toscano):

Vitolarsen Lots 23 ((b) (4) manufacturing method) and 57 ((b) (4) manufacturing method) were negative Ames and in vitro chromosomal aberration assays. Lot 23 was negative in a in vivo mouse bone marrow micronucleus assay; Lot 57 was not evaluated for in vivo clastogenicity.

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: A Bacterial Reverse Mutation Test of NS-065/NCNP-01 (NDK-65)-(3)

Study no.: TX10838
 Study report location: EDR
 Conducting laboratory and location:  (b) (4)

Date of study initiation: September 4, 2015
 GLP compliance: Yes (Japanese)
 QA statement: Yes
 Drug, lot #, and % purity: NS-065, Lot 63, 99.3%

Methods

Strains: TA100, TA1535, TA98, TA1537, WP2uvrA
 Concentrations in definitive study: 0, 156, 313, 625, 1250, 2500, 5000 µg/plate
 (+/- phenobarbital and 5,6-benzoflavone-induced rat liver S9 fraction)

Basis of concentration selection: Limit dose

Negative control: Saline

Positive control:

Strain	-S9	+S9
TA100	AF-2	2AA
TA1535	NaN ₃	2AA
WP2uvrA	AF-2	2AA
TA98	AF-2	2AA
TA1537	9AA	2AA

Formulation/Vehicle: Saline

Incubation & sampling time: 48 h

Results

Vitolarsen Lot 63 () manufacturing method) was negative in a GLP-compliant Ames assay.

***In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)**

Study title: A Bacterial Reverse Mutation Test of NDK-4001

Study no.: TX10986
 Study report location: EDR
 Conducting laboratory and location: [redacted] (b) (4)

Date of study initiation: December 3, 2018
 GLP compliance: Yes (Japan)
 QA statement: Yes
 Drug, lot #, and % purity: NDK-4001 (NDK-65), Lot 6, 98%;
 [redacted] (b) (4) impurities were present at [redacted] (b) (4)

Methods

Strains: TA100, TA1535, TA98, TA1537, WP2uvr
 Concentrations in definitive study: 0, 0.625, 1.25, 2.5, 5, 10, and 20 mg/plate
 NDK-65 (+/- phenobarbital and 5,6-benzoflavone-induced rat liver S9 fraction)
 Basis of concentration selection: 20 mg/plate was selected as the high dose to maximize the concentration of multimers
 Negative control: Saline
 Positive control:

Strain	-S9	+S9
TA100	4NQO	2AA
TA1535	NaN ₃	2AA
WP2uvrA	4NQO	2AA
TA98	4NQO	2AA
TA1537	9AA	2AA

Formulation/Vehicle: Saline
 Incubation & sampling time: 48 h

Results

Vitolarsen concentrations that were intended to maximize the amount of [redacted] (b) (4) impurities in the test systems were negative in a GLP-compliant Ames assay.

7.2 *In Vitro* Assays in Mammalian Cells

Study title: A Chromosomal Aberration Test of NS-065/NCNP-01 (NDK-65) in Cultured Mammalian Cells – (3)

Study no.: TX10839
Study report location: EDR
Conducting laboratory and location:  (b) (4)

Date of study initiation: September 10, 2015
GLP compliance: Yes (Japanese)
QA statement: Yes
Drug, lot #, and % purity: NS-065, Lot 63, 99.3%

Methods

Cell line: CHL/IU
Concentrations in definitive study: 0, 1250, 2500, 5000 µg/mL (+/- phenobarbital and 5,6-benzoflavone-induced rat liver S9 fraction)
Basis of concentration selection: Limit dose
Negative control: Saline
Positive control: Mitomycin c (-S9), cyclophosphamide (+S9)
Formulation/Vehicle: Saline
Incubation & sampling time: 6 and 18 h (+/- S9), 24 h (- S9),

Results

Vitolarsen Lot 63 ( manufacturing method) was negative in a GLP-compliant chromosomal aberration assay.

Study title: A Chromosomal Aberration Test of NDK-4001 in Cultured Mammalian Cells

Study no.: TX10987
 Study report location: EDR
 Conducting laboratory and location: (b) (4)

Date of study initiation: December 12, 2018
 GLP compliance: Yes (Japan)
 QA statement: Yes
 Drug, lot #, and % purity: NDK-4001 (NDK-065), Lot 6, 98%;
(b) (4) impurities were present at (b) (4)

Methods

Cell line: CHL/IU
 Concentrations in definitive study: 0, 2500, 5000, 10000 µg/mL NDK-065 (+/- phenobarbital and 5,6-benzoflavone-induced rat liver S9 fraction)
 Basis of concentration selection: No decreases in cell proliferation ratio were achieved; the high dose was selected to maximize exposure to the (b) (4) impurities.
 Negative control: Saline
 Positive control: Mitomycin c (-S9), cyclophosphamide (+S9)
 Formulation/Vehicle: Saline
 Incubation & sampling time: 6 h (+/-S9), 24 h (-S9)

Results

Vitolarsen concentrations that were intended to maximize the amount of (b) (4) in the test system were negative in a GLP-compliant chromosomal aberration assay.

9 Reproductive and Developmental Toxicology*Juvenile Animal Range-finding Studies*

Male CD-1 mice (5/group) were administered subcutaneous doses of 0, 60, 240, or 1000 mg/kg Vitolarsen of PND7 and 14, and IV bolus doses on PND 21 and 28. All main study mice were euthanized on PND29, while TK animals were euthanized on PND 7, 14, 21, or 28. Mortality, clinical signs, body weight, clinical chemistry parameters, macroscopic changes, kidney weights, and kidney histopathology were

evaluated. There was no mortality at any dose; however, mean relative (to BW) kidney weights were increased in HDM, and granular basophilic material was observed in the tubular epithelium in MDM and HDM.

Kidney Findings	Dose (mg/kg)			
	0	60	240	1000
Absolute Weight (g)	0.40	0.35	0.39	0.45
% BW	1.6	1.5	1.6	1.7
Basophilic granules	0	0	5	5
minimal	0	0	5	0
mild	0	0	0	5

To further assess the toxicity of vitolarsen, the study was repeated with doses of 0, 60, 240, 1000, or 2000 mg/kg administered by subcutaneous injection (PND7) or IV bolus (PNDs 14, 21, and 28). There was no drug-related mortality. At necropsy, drug-related findings included “tan focus” of the kidney in 2/5 HDM, correlating with tubular dilation, and increases in absolute and mean kidney weights at 1000 and 2000 mg/kg that correlated with renal tubule degeneration and necrosis, tubular dilation, basophilic material, and hyaline cases.

Kidney Findings	Dose (mg/kg)				
	0	60	240	1000	2000
Tan Foci	0	0	0	0	2
Absolute Weight (g)	0.34	0.36	0.41	0.42	0.47
% BW	1.5	1.4	1.5	1.5	1.8
Basophilic granules	0	0	4	5	5
Tubular necrosis	0	0	0	4	5
Intratubular basophilic material	0	0	0	5	5
Hyaline cast	0	0	0	2	4
Tubular dilation	0	0	0	5	5

Study title: A 10 Week Subcutaneous and Intravenous Study of NS-065/NCNP-01 in Juvenile Mice, Including a 10 Week Recovery Period

Study no.: TX10844
Study report location: EDR
Conducting laboratory and location:  (b) (4)
Date of study initiation: September 29, 2017
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: NS-065, Lot 60, 100.9%

Methods

Doses: 0, 15, 60, 240, 1200 mg/kg
Frequency of dosing: Weekly
Route of administration: SC (initial dose), IV (remaining doses)
Dose volume: 10 mL/kg
Formulation/Vehicle: Saline
Species/Strain: CD1 Mice
Number/Sex/Group: 40 males/group (main and recovery)
Age: PND 7 at initiation of dosing
Weight: 1.3 to 4.5 g
Satellite groups: TK (33/Group)
Unique study design: Only male animals were evaluated. Dosing was initiated on PND 7 by SC injection, while all subsequent doses were by IV injection; offspring were weaned on PND21.
Deviation from study protocol: None

Observations and Results**Mortality and Clinical Signs**

Litters were monitored twice daily for mortality during the preweaning period. After weaning, animals were monitored twice daily for mortality or signs of morbidity. There was no drug-related mortality at doses up to 240 mg/kg. COD for 5 of the 8 deaths in HDM was thought to be drug-related kidney toxicity (i.e., tubular degeneration), and occurred between PND 24 and 70. Drug-related clinical signs were observed in HDM, and included partly closed eyes (28/40), decreased activity (20/40), and erected fur (31/40).

Mode of Death	Dose Group														
	Control			15 mg/kg			60 mg/kg			240 mg/kg			1200 mg/kg		
	M	R	TK	M	R	TK	M	R	TK	M	R	TK	M	R	TK
Unknown Cause of Death	0	0	0	0	1 (3405)	0	1 (504)	1 (2608)	0	0	0	0	0	0	0
Procedural (Blood Collection)	0	0	0	0	0	0	0	0	1 (4810)	0	0	0	0	0	2 (5608, 5702)
Incorrect Sex	0	0	0	0	0	0	0	0	0	1 (206)	0	0	0	0	0
Cannibalized	0	0	0	0	0	0	0	0	0	0	1 (3803)	0	0	1 (2407)	0
Test Article-Related	0	0	0	0	0	0	0	0	0	0	0	0	1 (1007)	4 (2810, 3101, 3104, 3701)	0
Total	0	0	0	0	1	0	1	1	1	1	1	0	1	5	2

M = Main Study; R = Recovery Phase; TK = Toxicokinetic Phase

(Sponsor's Table)

Body Weights and Food Consumption

Body weights were evaluated weekly during the preweaning period, and twice weekly after weaning. Food consumption was monitored twice weekly after weaning. Mean body weight gain in HDM was decreased relative to controls by 3 to 7% and 5 to 8% in dosing and recovery periods, respectively. There were no drug effects on food consumption.

Ophthalmoscopy

Examinations by indirect ophthalmoscopy were conducted during the last week of the dosing and recovery periods. There were no drug-related effects.

ECG

Not evaluated.

Hematology, Clinical Chemistry, and Urinalysis

Blood and urine samples were collected from fasted animals at scheduled necropsy (PND 71 and 141). There were no drug effects on hematology, clinical chemistry, or urinalysis parameters.

Gross Pathology and Organ Weights

There were no drug-related gross findings at scheduled necropsy in main study animals. However, abnormal kidney appearance was observed in 2/40 HDM in the recovery group. Absolute and relative (to BW) prostate and thymus weights were decreased in main study animals; there were no drug-related effects on organ weight in

the recovery groups. There were no drug effects on brain weight or morphometric parameters.

	Males			
Group	2	3	4	5
Dose (mg/kg)	15	60	240	1200
No. Animals per Group	20	19	19	19
Prostate (No. Weighed)^a	20	19	19	19
Absolute value	-23.85	-18.37	-27.20	-31.61
% of body weight	-22.86	-16.90	-23.21	-30.68
Thymus (No. Weighed)	20	19	19	19
Absolute value	-4.74	1.49	-10.49	-22.58
% of body weight	-3.44	1.41	-6.06	-21.35

^a All values expressed as percent difference of control group means.

Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – $P \leq 0.05$ or $P \leq 0.01$; refer to data tables for actual significance levels and tests used.

(Sponsor's Table)

Histopathology

Adequate Battery: A complete histopathology panel, including an expanded neurohistopathology panel, was evaluated in control and HDM after dosing and recovery periods.

Tissue Panel

Aorta	Thyroid gland	Pancreas
Bone (femur and sternum)	Gross lesions	Injection site
Bone marrow (femur and sternum)	GALT	Skin
Brain	Heart	Duodenum
Epididymis	Kidneys	Ileum
Esophagus	Cecum	Jejunum
Eyes	Colon	Spinal cord
Gallbladder	Rectum	Spleen
Adrenal gland	Liver	Stomach
Harderian gland	Lungs	Testis
Parathyroid gland	Mandibular LN	Thymus
Pituitary gland	Mesenteric LN	Tongue
Prostate gland	Skeletal muscle	Trachea
Salivary gland	Optic nerve	Urinary bladder
Seminal vesicle	Sciatic nerve	

Tissues	Tissues
Olfactory bulb glomerular mitral and granular cell layers	Pontine nuclei
Frontal, parietal and temporal cortex	Medial geniculate nucleus
Cingulate and retrosplenial cortex	Posterior collicular nuclei
Septal nucleus	Lateral anterior olivary nucleus
Piriform cortex	Red nucleus
Anterior commissure	Substantia nigra
Bed nucleus stria terminalis	Raphe nuclei
Caudate, putamen and globus pallidus	Cerebellar lobes vermis, ansiform and floccular-nodular
Internal capsule	Cerebellar roof nuclei
Amygdaloid nucleus	Facial nucleus
Thalamus	Genu of facial nerve
Hypothalamus	Medial and Lateral vestibular nuclei
Habenular nuclei	Reticular gray matter
Subiculum	Posterior olivary nuclei
Hippocampal sites CA1,2 &3	Trigeminal motor nucleus and spinal sensory tract
Hippocampal dentate gyrus	Cuneate and gracile nuclei
Anterior colliculi	Hypoglossal nucleus
Cerebral peduncle	Pyramids

(Sponsor's table; subanatomic brain tissues)

Signed Pathology Report: Yes

Peer Review: No

Histological Findings: Drug-related findings in main study animals included signs of kidney toxicity (tubular degeneration, basophilia, and vacuolation), as well as injection site inflammation and lymphoid depletion in the thymus. Signs of drug-related kidney toxicity were also observed in recovery animals. Evaluation of the expanded neurohistopathology panel did not indicate any drug effects on neurodevelopment.

	Males				
Group	1	2	3	4	5
Dose (mg/kg)	0	15	60	240	1200
No. Animals Examined	20	20	19	19	19
Kidney (No. Examined)	20	20	19	19	19
Degeneration, tubular	(0)	(0)	(0)	(4)	(19)
Minimal	0	0	0	4	0
Mild	0	0	0	0	9
Moderate	0	0	0	0	9
Marked	0	0	0	0	1
Basophilia, tubular	(0)	(0)	(0)	(12)	(19)
Minimal	0	0	0	7	1
Mild	0	0	0	5	4
Moderate	0	0	0	0	8
Marked	0	0	0	0	6
Vacuolation, tubular	(0)	(0)	(0)	(10)	(19)
Minimal	0	0	0	5	2
Mild	0	0	0	4	5
Moderate	0	0	0	1	10
Marked	0	0	0	0	2
Hypertrophy, tubular	(0)	(3)	(4)	(2)	(6)
Minimal	0	3	4	2	6
Injection Site, Tail Vein (No. Examined)	20	20	19	19	19
Inflammation, mixed cell	(1)	(3)	(8)	(7)	(6)
Minimal	1	3	8	7	6
Thymus (No. Examined)	20	0	0	0	19
Depletion, lymphoid	(1)	0	0	0	(6)
Minimal	1	0	0	0	3
Mild	0	0	0	0	3

(Sponsor's Table; PND 71)

Group	Males				
	1	2	3	4	5
	Dose (mg/kg)	0	15	60	240
No. Animals Examined	20	20	19	20	18
Kidney (No. Examined)	20	0	19	20	18
Degeneration, tubular	(0)	-	(0)	(3)	(16)
Minimal	0	-	0	3	7
Mild	0	-	0	0	8
Moderate	0	-	0	0	1
Basophilia, tubular	(0)	-	(0)	(0)	(14)
Minimal	0	-	0	0	10
Mild	0	-	0	0	4
Vacuolation, tubular	(0)	-	(0)	(0)	(14)
Minimal	0	-	0	0	10
Mild	0	-	0	0	4
Nephropathy, chronic progressive	(0)	-	(2)	(5)	(11)
Minimal	0	-	2	5	7
Mild	0	-	0	0	3
Moderate	0	-	0	0	1
Hypertrophy, tubular	(2)	-	(1)	(4)	(5)
Minimal	2	-	1	4	5

- = not applicable

(Sponsor's Table; PND 141)

Special Evaluation

Sexual Maturation

Preputial separation was evaluated daily beginning on PND22. The mean day of preputial separation was 28.4 days for HDM, and 27.5 days in control; however, it was unclear if this was a drug-related effect given the laboratory's historical range of 26.9 to 33.6 days.

Neurobehavioral Assessments

Performance in the FOB, open field, acoustic startle, and Morris water maze tests was evaluated at the end of the dosing and recovery periods. Separate sets of animals (20/group) were used at each timepoint. There were no drug effects on neurobehavioral parameters.

Bone density and length

Femur length and density were measured on PND 71 and 141; there were no drug effects.

Toxicokinetics

Anti-drug antibodies were not detected in any group. TK parameters were analyzed on PND 14 and 70. Increases in C_{max} and were dose proportional on Day 14 and less-than

dose proportional on Day 70. Increases in AUC were dose-proportional on Days 14 and 70.

Parameter	Day	Dose (mg/kg)			
		15	60	240	1200
T _{max} (h)	14	0.1	0.1	0.1	0.1
	70	0.1	0.1	0.1	0.1
C _{max} (µg/mL)	14	101.4	355.0	1766	8483
	70	64.88	253.9	1062	1988
AUC _{0-24h} (µg*h/mL)	14	203.5	716.4	4340	21030
	70	130.6	542.4	2144	8970

Dosing Solution Analysis

Dosing solutions were within 15% of their respective target concentrations.

9.1 Fertility and Early Embryonic Development

Study title: Study for Effects of NS-065/NCNP-01 (NDK-65) on Fertility and Early Embryonic Development to Implantation by Intermittent Intravenous Administration in Male Mice

Study no.: TX10958
 Study report location: EDR
 Conducting laboratory and location:



Date of study initiation: January 22, 2018
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: NS-065, Lot 72, 94.5%

Methods

Doses:	0, 60, 240, 1000 mg/kg
Frequency of dosing:	Weekly
Dose volume:	10 mL/kg
Route of administration:	IV
Formulation/Vehicle:	Saline
Species/Strain:	CD-1 mice
Number/Sex/Group:	25
Satellite groups:	TK arm
Study design:	Males were dosed weekly beginning 9 weeks prior to mating, and through the mating period until the day before necropsy. Females were not administered NS-065. Mating was conducted between Days 63 and 76. Males were necropsied on Day 78. Females were necropsied on GD13.
Deviation from study protocol:	No significant deviations

Observations and Results**Mortality and Clinical Signs**

Animals were observed once daily (non-dosing) or 3 times daily (dosing) for mortality, morbidity, and clinical signs; there were no drug-related effects.

Body Weight and Food Consumption

Body weight and food consumption were evaluated every 3 or 4 days. There were no adverse effects on body weight or food consumption.

Toxicokinetics

Increases in C_{max} were dose-proportional, and increases in AUC were greater than dose proportional. Anti-drug antibodies were not detected.

Day of dosing	Dose level (mg/kg)	C_{max} ($\mu\text{g/mL}$)	T_{max} (h)	AUC_{0-24h} ($\mu\text{g}\cdot\text{h/mL}$)
0	60	246.1	0.1	67.46
	240	804.3	0.1	464.6
	1000*	3999	0.1	6432
63	60	143.1	0.1	57.09
	240	781.0	0.1	453.4
	1000	3717	0.1	7283

(Sponsor's Table)

Dosing Solution Analysis

Dosing solutions ranged from 95.6 to 101.8% of their respective target concentrations.

Necropsy

There was an approximate 2-fold increase in mean BUN in HDM relative to control. In males, there were no drug-related gross findings or effects on sperm parameters. Following cesarean section in females, there were no drug effects on corpora lutea, implantations, implantation rate, pre or postimplantation loss, viability, or live or dead embryos.

10 Special Toxicology Studies

Assessment of Manufacturing Changes

The sponsor conducted two, 5-week studies to compare the toxicity of vitolarsen lots using (b) (4) or (b) (4) manufacturing methods. In both studies, male cynomolgus monkeys (5/group) were administered 0, 200, or 600 mg/kg vitolarsen by weekly IV injection for 5 weeks. Study TX10832 compared vitolarsen (b) (4) (Lot 59) and (b) (4) (Lot 57) methods, and Study TX10837 compared vitolarsen (b) (4) (Lot 57) and (b) (4) (Lot 63) methods. There were no differences in adverse effects or drug exposure between lots manufactured by the (b) (4) or (b) (4) methods.

Finding	Study TX10832 (mg/kg)					Study TX10837 (mg/kg)				
	0	(b) (4) 200	(b) (4) 200	(b) (4) 600	(b) (4) 600	0	(b) (4) 200	(b) (4) 200	(b) (4) 600	(b) (4) 600
Urine Protein (mg/dL)	0.56	2.08	1.86	2.10	3.64	2.38	3.14	2.42	4.32	1.34
BUN (mg/dL)	19.92	22.16	20.94	27.78	30.64	24.96	21.88	24.16	29.48	27.00
Basophilic Granules	0/5	5/5	5/5	5/5	5/5	0/5	2/5	3/5	4/5	5/5
Vacuolation	0/5	0/5	0/5	1/5	3/5	0/5	0/5	0/5	4/5	1/5
Tubule Dilatation	0/5	0/5	0/5	1/5	2/5	0/5	0/5	0/5	1/5	1/5
Day 28: C _{max} (µg/mL)	N/A	2563	2319	6897	7346	N/A	2324	2348	7200	7507
Day 28: AUC _{0-24h} (µg*h/mL)	N/A	910	1035	3796	4777	N/A	1070	1018	4623	4347

Assessment of (b) (4) Impurities (Study TX10974)

The toxicity of (b) (4) impurities was assessed in male cynomolgus monkeys (5/group), in which heat-stressed or non-heat stressed vitolarsen (0, 200, 600 mg/kg) was administered weekly for 13 weeks. Adverse effects included increases in BUN, increases in absolute kidney weight, and focal dilatation of the kidney tubules in HDM, and epithelial vacuolation and increases in C-reactive protein in MDM and HDM. The

severity of the drug-related findings was consistent between non-stressed and stressed batches, and there was no additional toxicity associated with the stressed batch. TK parameters for vitolarsen were also consistent between non-stressed and stressed batches.

Finding	Non-Stressed (mg/kg)			Stressed (mg/kg)	
	0	200	600	200	600
CRP (mg/dL)	0.576	0.640	0.824	0.700	0.786
BUN (mg/dL)	20.20	20.74	34.06	21.14	30.88
Abs. Kidney Weight (g)	11.38	12.70	15.04	11.82	15.36
Cysts	0/5	0/5	2/5	0/5	0/5
Vacuolation	0/5	2/5	5/5	1/5	5/5
Tubule Dilation	0/5	0/5	3/5	0/5	5/5
Day 85: C _{max} (µg/mL)	N/A	2570	7704	2602	6970
Day 85: AUC _{0-24h} (µg*h/mL)	N/A	1152	4969	1256	4659

Dose Range-Finding Studies to Support Planned Carcinogenicity Studies

Dose-ranging studies to support the planned carcinogenicity studies in Tg.rasH2 mice and Wistar rats were reviewed under SPA1 (July 13, 2018) and SPA2 (August 9, 2019), respectively, by C. Toscano, submitted to IND 127474.

To support the planned 26-week study in TgRasH2 mice, the sponsor conducted a 4-week study in male CByB6F1-Tg(HRAS)2Jic, wild type mice administered 0, 250, 500, or 1000 mg/kg NS-065 by weekly IV bolus. The NOAEL was 500 mg/kg due to renal tubule dilation and BUN elevation. The Executive CAC issued the following recommendations to the sponsor (July 12, 2018):

- The Committee recommended doses of 0 (vehicle), 50, 150, and 500 mg/kg/day, with the high dose based on increases in BUN and histopathology changes observed at 1000 mg/kg/day in the 4-week dose-ranging study.
- The mid and low doses were selected to provide a dose-response based on plasma exposure (AUC).
- Because the study is to be conducted only in males, the number of animals per group should be increased to 50/group.
- The Committee noted that toxicokinetic analysis in this transgenic mouse study is not needed for the FDA.
- If there are survival issues during the study, the sponsor should contact the Review Division before any changes are made.

To support the planned 2-year study, the sponsor conducted a 4-week study in which 0, 100, 300, or 1000 mg/kg vitolarsen was administered by weekly IV bolus to male Wistar (5/group) rats. Based on findings of renal tubule dilation in HDM, a 13-week range-

finding study was the conducted in male Wistar rats administered 0, 250, 500, or 1000 mg/kg vitolarsen by weekly IV bolus. However, due to tubule dilation at all doses in the 13-week study, a NOAEL was not defined. The Executive CAC issued the following recommendations to the sponsor (August 9, 2019):

- The Committee recommended weekly IV doses of 0 (0.9% w/v saline), 25, 80, and 250 mg/kg, with the high dose based on kidney findings (tubular dilation, vacuolation, and basophilia and increases in urinary protein) at higher doses in the 13-week dose-ranging study.
- The mid and low doses were selected to achieve adequate plasma exposure (AUC) spacing.
- If there are survival issues during the study, the Sponsor should contact the Review Division before any changes are made.

11 Integrated Summary and Safety Evaluation

Introduction

Vitolarsen is a morpholino antisense oligonucleotide developed by Nippon Shinyaku Co., Ltd. for the treatment of Duchenne Muscular Dystrophy (DMD). Vitolarsen is intended to induce skipping of exon 53 in the human dystrophin gene, thus resulting in production of a truncated but potentially functional dystrophin protein.

Pharmacology

Primary Pharmacology

Proof of concept studies for vitolarsen were conducted in *mdx* and *mdx52* mice, which harbor a point mutation leading to a stop codon in exon 23 or lack exon 52 of the dystrophin gene, respectively. In male *mdx* mice, in situ hybridization imaging indicated localization of vitolarsen in the quadriceps 1 h after an IV dose of 500 mg/kg. In male *mdx52* mice, IV administration of a mouse surrogate (NDK-73) as well as an additional morpholine oligonucleotide (NDK-33), which targets the Exon 53 3' end of the mouse dystrophin gene, resulted in 4.8% skipping efficiency in the gastrocnemius muscle 2 weeks after administration of 1280 mg/kg combination (640 mg/kg of each oligonucleotide).

In cynomolgus monkeys, exon skipping efficiency was 1.3 and 5.4% following weekly IV administration of 200 and 600 mg/kg vitolarsen, respectively. In the same study, dose-dependent increases in exon skipping efficiency reached 37.1% in gastrocnemius muscle. No exon skipping was detected in cardiac muscle in a similar 12-week study in which 0 or 100 mg/kg vitolarsen was administered by IM injection; however, 8.6 and 13.2% skipping efficiency was seen in quadriceps muscle at the end of the dosing and recovery periods, respectively. In the 39-week toxicology study, weekly administration of up to 360 mg/kg vitolarsen resulted in approximately 6 and 4% exon skipping efficiency in skeletal and cardiac muscle at the end of the dosing period, and approximately 5 and 1% skipping efficiency, respectively, after the 8-week recovery period.

Potential effects in humans were evaluated in using isolated human rhabdomyosarcoma cells, in which the EC₅₀ for exon 53 skipping by vitolarsen ranged from 3.4 to 5.9 nM. Additionally, 83.1% exon 53 skipping efficiency was observed 1 week after transfection of myoblasts derived from DMD patient fibroblasts with 10 uM vitolarsen, correlating with expression of a presumably truncated dystrophin protein.

Secondary Pharmacology

Sequence analysis of vitolarsen and n+, n-1, or n-2 oligomers indicated a possible off-target interaction with 19 genes (ALDH1A2, APCDD1, CAMKK2, CNTNAP2, FSHR, FUT1, LMTK2, LRIG1, MYT1, PCDH15, PRKCH, RP11-459O1.2, RP11-479O16.1, SLC22A10, SLC24A2, TIAM1, WDR20, WRN, ZNF557). In vitro gene expression studies in HEK293 and ITO-II cells indicated the potential for drug-mediated changes in mRNA levels for APCDD1, CNTNAP2, FUT1, and MYT1. Observations in a clinical trial (Study P1/2) indicated hair discoloration in one subject administered vitolarsen. Although such an effect might indicate an off-target effect on APCDD1 expression, concern over such a possibility is minimal given the intended indication (i.e., DMD).

Safety Pharmacology

Dedicated single dose studies in rat (CNS) and monkey (cardiovascular and respiratory) did not indicate drug effects on safety pharmacology endpoints, and there was no effect on QT prolongation in CHO cell cultures exposed to up to 3 mg/mL vitolarsen.

Toxicology

General Toxicology

Repeat-dose studies in adult male CD1 mice, and single and repeat-dose studies in adult male cynomolgus monkeys were conducted to evaluate the general toxicology of vitolarsen.

Repeat-dose toxicology studies in adult male mice included 13- and 26-week studies in which weekly IV administration of 0, 60, 240, or 1000 mg/kg and 0, 15, 60, 240, or 1000 mg/kg vitolarsen, respectively, was evaluated. The primary toxicity in both studies was renal tubule injury. Kidney toxicity in the pivotal study, which resulted in unscheduled death of two HDM, was characterized by slight increases in urea nitrogen and creatinine, increases in kidney weight in HDM, and histologic findings of intratubular dilation and epithelial vacuolation in the distal tubule and collecting duct in MDM and HDM. Histology findings did not resolve over an 8-week recovery period. Based on the kidney findings in the 26-week study, the NOAEL for IV administration of vitolarsen in male CD1 mice was 60 mg/kg ($C_{\max} = 210.2 \mu\text{g/mL}$; $\text{AUC}_{0-24\text{h}} = 67.47 \mu\text{g}\cdot\text{h/mL}$).

In adult male monkeys, single IV doses up to 600 mg/kg resulted in vacuolation of the proximal renal tubules; however, there was no renal toxicity following single, IM doses up to 100 mg/kg vitolarsen. Repeat (weekly) IV dosing in monkey was assessed in 12- and 39-week studies at doses of 0, 60, 200, or 600 and 0, 10, 60, or 360 mg/kg vitolarsen, respectively. In both studies, the primary toxicity was epithelial vacuolation and dilation of the proximal tubules in HDM. Based on the 39-week study, the NOAEL for IV administration of vitolarsen in male cynomolgus monkeys was 60 mg/kg ($C_{\max} = 613.9 \mu\text{g/mL}$; $\text{AUC}_{0-24\text{h}} = 261.1 \mu\text{g}\cdot\text{h/mL}$).

To support dosing in pediatric patients, juvenile male CD1 mice were administered 0, 15, 60, 240, or 1200 mg/kg vitolarsen by SC injection on PND 7 and weekly IV injection from PND 14 to 70. Primary toxicity included renal tubule vacuolation, dilation, and degeneration at doses above 60 mg/kg in dosing and recovery groups; renal toxicity was determined to be the COD for 5 HDM. There were no correlating clinical chemistry or urinalysis parameters. There were no drug-effects on neurobehavioral, sexual maturation, or bone density parameters. Based on kidney toxicity, the NOAEL for IV administration of vitolarsen in juvenile mice was 60 mg/kg ($C_{\max} = 253.9 \mu\text{g/mL}$; $\text{AUC}_{0-24\text{h}} = 542.4 \mu\text{g}\cdot\text{h/mL}$).

Fertility and Early Embryonic Development

An assessment of reproductive and developmental toxicity was limited to a fertility and early embryonic development study in male CD1 mice. Weekly IV injection of 0, 60, 240, or 1000 mg/kg vitolarsen was initiated 9 weeks prior to mating with control female

animals (Days 63 and 76), followed by necropsy of the females on GD 13. There were no drug effects on corpora lutea, implantations, implantation rate, pre or postimplantation loss, viability, or live or dead embryos.

Genetic Toxicology

Ames, in vitro chromosomal aberration, and mouse bone marrow micronucleus assays did not indicate any genotoxic potential for vitolarsen.

Additional Studies

The sponsor conducted additional genetic toxicology and general toxicology studies to assess the safety of (b) (4) impurities as well as any differences between batches manufactured using (b) (4) or (b) (4) manufacturing methods.

The (b) (4) impurities were qualified by comparing stressed and non-stressed batches of vitolarsen. Based on discussion with the CMC team, the levels of (b) (4) impurities in the stressed batch exceeded those that would be expected in the clinical formulation. The stressed drug batch was negative in Ames and in vitro chromosomal aberration assays, and there was no additional toxicity or potentiation of existing toxicity when compared with a non-stressed batch of vitolarsen in a 13-week general toxicology study in cynomolgus monkeys. Based on the results of the sponsor's genetic and general toxicology studies of (b) (4) impurities, as well as discussion with the CMC team, the potential toxicity of the (b) (4) impurities has been adequately assessed.

Vitolarsen manufactured using the (b) (4) method was negative in Ames, in vitro chromosomal aberration, and in vivo mouse bone marrow nucleus assays. Additionally, vitolarsen batches manufactured using the (b) (4) and (b) (4) methods were negative in Ames and chromosomal aberration assays but were not evaluated in an in vivo clastogenicity assays. However, discussion with the CMC team has indicated a similar impurity profile between all three manufacturing methods, with the (b) (4) method resulting in the lowest levels of impurities. A comparison of general toxicity did not indicate any differences in toxicity between the manufacturing methods. Based on the sponsor's nonclinical studies comparing the toxicity of the (b) (4) (b) (4) and (b) (4) as well as discussion with the CMC team indicating similar impurity profiles, any batch differences resulting from the described changes in the manufacturing process are qualified.

Summary and Conclusion

The sponsor's general and juvenile animal toxicology studies indicated a risk for drug-related kidney injury, but such effects are likely monitorable in a clinical setting. Additional nonclinical studies did not indicate genotoxic potential or risk for male reproductive function. Carcinogenicity was not evaluated but is to be assessed as a postmarketing requirement.

The nonclinical data support approval vitolarsen.

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/s/

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