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)m 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 Cmax 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-1β, 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. Vitolarsen 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} (μg/mL)	137.6	134.6	115.1	122.8	805.9	700.3	598.6	613.9	3665	3522	4198	3711
$AUC_{0\text{-}24h}$ (μg*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 ((b) (4) 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: (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%; (b) (4) impurities were present at (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 (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 ((b) (4) 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 µgg/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 o (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 weening, 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 weening. Food consumption was monitored twice weekly after weening. 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)

	Males				
Group	**1**	**2**	**3**	**4**	**5**
Dose (mg/kg)	**0**	**15**	**60**	**240**	**1200**
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: (b) (4)
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} (µg/mL)	T_{max} (h)	AUC_{0-24h} (µg·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) (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	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 Dilation	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) (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 dilation 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: $AUC_{0\text{-}24h}$ (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 EC_{50} 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 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 µg/mL; AUC_{0-24h} = 67.47 µg*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 µg/mL; AUC_{0-24h} = 261.1 µg*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 µg/mL; AUC_{0-24h} = 542.4 µg*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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