Clinical Study Protocol: NS-065/NCNP-01-202

Study Title:	A Phase II, Open-Label, Extension Study to Assess the Safety and Efficacy of NS-065/NCNP-01 in Boys with Duchenne Muscular Dystrophy (DMD)
Protocol Number:	NS-065/NCNP-01-202
Study Phase:	Phase II
Product Name:	NS-065/NCNP-01 Injection
IND Number:	127,474
Investigators:	Up to 6 clinical sites located in North America
Sponsor:	NS Pharma, Inc. 140 E Ridgewood Avenue, Suite 280S Paramus, NJ 07652
Study Chair:	Paula R. Clemens, MD Professor of Neurology University of Pittsburgh
Sponsor Medical Monitor:	Helmut Albrecht, MD, MS, FFPM PharmaLex Development Services, LLC
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Confidentiality Statement

STUDY SYNOPSIS

Study Title	A Phase II, Open-Label, Extension Study to Assess the Safety and Efficacy of NS-065/NCNP-01 in Boys with Duchenne Muscular Dystrophy (DMD)
Protocol Number	NS-065/NCNP-01-202
Name of Sponsor	NS Pharma, Inc.
Investigative Product	NS-065/NCNP-01 (viltolarsen) Injection
Study Phase	Phase II
Indication	Treatment of Duchenne muscular dystrophy (DMD)
Objectives	Primary: 1. To evaluate the safety and tolerability of low (40 mg/kg/week) and high (80 mg/kg/week) intravenous (IV) doses of NS-065/NCNP-01 Injection up to 192-week treatment period (up to a total of 216 weeks of treatment in Study NS-065/NCNP-01-201 and 202), in boys with DMD that completed Study NS-065/NCNP-01-201. 2. To investigate the effect of low and high IV doses of NS-065/NCNP-01 Injection after up to 192-week Treatment Period (up to a total of 216 weeks of treatment in Study NS-065/NCNP-01-201 and 202) on muscle strength, mobility, and functional exercise capacity, as measured by Time to Stand (TTSTAND) in boys with DMD that completed Study NS-065/NCNP-01-201 vs. matched DMD historical controls in boys with DMD. Secondary: 1. To investigate the effect of low and high IV doses of NS-065/NCNP-01 Injection after up to 192-weeks treatment (up to a total of 216 weeks of treatment in Study NS-065/NCNP-01-201 and 202) on muscle strength, mobility, and functional exercise capacity, as measured by Time to Run/Walk 10 meters (TTRW), Time to Climb 4 stairs (TTCLIMB), North Star Ambulatory Assessment (NSAA), Six-minute Walk Test (6MWT), and Quantitative Muscle Testing (QMT) in boys with DMD that completed Study NS-065/NCNP-01-201 vs. matched historical controls in boys with DMD. Exploratory: 1. To investigate the effects of low and high IV doses of NS-065/NCNP-01 Injection on serum pharmacodynamic (PD) biomarkers.
Study Design	This is a Phase II, multicenter, open-label, extension study of NS-065/NCNP-01 Injection 250 mg administered intravenously once weekly for up to 192-weeks (up to a total of 216 weeks of treatment in Study NS-065/NCNP-01-201 and 202) to boys with DMD who complete Study NS-065/NCNP-01-201. This study will evaluate the safety, tolerability, and clinical efficacy of NS-

	065/NCNP-01 Injection 250 mg at dose levels of up to 80 mg/kg/week administered by weekly IV infusion over an additional treatment period of up to 192-weeks (up to a total of 216 weeks of treatment in Study NS-065/NCNP-01-201 and 202) or until enrollment in a separate long-term follow up program of NS-065/NCNP-01, whichever is earlier. Week 193 is a comprehensive assessment at the beginning of the extension visits incorporated in protocol amendment 10, and Week 217 is the final study assessment for the treatment period ending with Week 216. Patients who complete the Phase II Dose-finding Study NS-065/NCNP-01-201 are eligible to enroll. Patients interested in enrolling in this extension study will be consented prior to or during the Week 24 study visit of the NS-065/NCNP-01-201 study. In order to accommodate patient recovery following the scheduled biopsy during the Week 25 of Study NS-065/NCNP-01-201, patients will be allowed a visit window of 7 days (+/- 3 days) from the Week 24 infusion to complete both the end of study procedures for study 201 and the first infusion in this extension study. All Week 25 study assessments, including muscle biopsy, of the NS-065/NCNP-01-201 study must be completed prior to first infusion in this extension study. Patients can receive the first infusion of this extension study on the same day as the Week 25 study (final) visit of the NS-065/NCNP-01-201 study, as long as all study assessments (including muscle biopsy) of the NS-065/NCNP-01-201 study are completed prior to infusion.
Study Population	**Inclusion Criteria:** 1. Patient's parent or legal guardian has provided written informed consent/HIPAA authorization prior to any extension study-specific procedures and patient has provided assent appropriate for his age and developmental status. 2. Patient completed Study NS-065/NCNP-01-201 through Week 25. 3. Patient and parent/guardian are willing and able to comply with scheduled visits, investigational product administration plan, and study procedures. 4. Patient must be on a stable dose of glucocorticoid (GC), and is expected to remain on the stable dose of GC treatment for the duration of the study. **Exclusion Criteria:** 1. Patient had a serious or severe adverse event in Study NS-065/NCNP-01-201 that, in the opinion of the Investigator and/or Sponsor, was probably or definitely related to NS-065/NCNP-01 Injection 250 mg use and precludes safe use of NS-065/NCNP-01 Injection 250 mg for the patient in this study. 2. Patient had a treatment which was made for the purpose of dystrophin or dystrophin-related protein induction after completion of Study NS-065/NCNP-01-201.

	3. Patient took any other investigational drugs after completion of Study NS-065/NCNP-01-201. 4. Patient was judged by the investigator and/or the Sponsor that it was not appropriate to participate in the extension study for other reasons.
Clinical Sites	Up to 6 clinical sites located in North America
Test Product, Dose, and Mode of Administration	NS-065/NCNP-01 Injection 250 mg aqueous infusions will be supplied as a 10-mL or 5-mL glass vial containing 25 mg/mL or 50 mg/mL of drug product in saline, respectively. Patients will begin dosing in this study at the same dose level they received in Study NS-065/NCNP-01-201. Once sites receive IRB approval of Protocol Amendment 10, all subjects who were previously in the low dose cohort of 40mg/kg/week will receive 80mg/kg/week after obtaining patient consent/assent. Patients will continue at dose level of up to 80 mg/kg/week for the remaining duration of the trial, up to 192-week (up to a total of 216 weeks of treatment in Study NS-065/NCNP-01-201 and 202) extension study or enrollment in a separate long-term follow up program of NS-065/NCNP-01, whichever is earlier. Investigational product will be prepared in accordance with an Investigational Product Instruction Manual (IPIM) and administered by intravenous infusion over a 1-hour period.
Safety Assessments	1. Overall incidence of adverse events (AEs) and serious adverse events (SAEs) 2. Changes from baseline compared to Screening Visit and Week 25 of Study NS-065/NCNP-01-201 in laboratory parameters (blood, urine). 3. Changes from baseline compared to Day 1 and Week 25 of Study NS-065/NCNP-01-201 in electrocardiograms (ECGs), anthropometrics, vital signs, and diagnostic parameters (physical exam). 4. Anti-NS-065/NCNP-01 antibodies at Weeks 37, 49, 73, 97, 121, 145, 169,193 and 217 compared to Day 1 and Week 24 of Study NS-065/NCNP-01-201. 5. Anti-dystrophin antibodies at Weeks 37, 49, 73, 97, 121, 145, 169, 193 and 217 compared to Day 1 and Week 24 of Study NS-065/NCNP-01-201. 6. Cytokine levels at Weeks 37, 49, 73, and 97 compared to Day 1 and Week 24 of Study NS-065/NCNP-01-201.
Efficacy Assessments	<u>Primary</u>: Time to Stand (TTSTAND) at 37, 49, 61, 73, 85, 97, 109, 121, 133, 145, 157,169, 181, 193, 205 and 217 Weeks compared to Pre-Infusion Visit and Week 25 of Study NS-065/NCNP-01-201. <u>Secondary</u>:

	1. Time to Run/Walk 10 meters (TTRW) at 37, 49, 61, 73, 85, 97, 109, 121, 133, 145, 157, 169, 181, 193, 205 and 217 Weeks compared to Pre-Infusion Visit and Week 25 of Study NS-065/NCNP-01-201 2. Time to Climb 4 stairs (TTCLIMB) at 37, 49, 61, 73, 85, 97, 109, 121, 133, 145, 157, 169, 181, 193, 205 and 217 Weeks compared to Pre-Infusion Visit and Week 25 of Study NS-065/NCNP-01-201 3. North Star Ambulatory Assessment (NSAA) at 37, 49, 61, 73, 85, 97, 109, 121, 133, 145, 157, 169, 181, 193, 205 and 217 Weeks compared to Pre-Infusion Visit and Week 25 of Study NS-065/NCNP-01-201 4. Six-minute Walk Test (6MWT) at 37, 49, 61, 73, 85, 97, 109, 121, 133, 145, 157, 169, 181, 193, 205 and 217 Weeks compared to Pre-Infusion Visit and Week 25 of Study NS-065/NCNP-01-201 5. Quantitative Muscle Testing (QMT) at 37, 49, 61, 73, 85, 97, 109, 121, 133, 145, 157, 169, 181, 193, 205 and 217 Weeks compared to Pre-Infusion Visit and Week 25 of Study NS-065/NCNP-01-201
Pharmacokinetic Assessments	Blood samples will be collected during the same visits when blood samples are collected for anti-NS-065/NCNP-01 and anti-dystrophin antibody analyses. PK samples will only be analyzed in the event that anti-NS-065/NCNP-01 antibodies are detected in order to understand the impact of immunogenicity on plasma levels of the drug.
Exploratory Assessments	Blood will be drawn for a serum PD biomarker panel to explore effect of NS-065/NCNP-01 on biomarkers of muscle cellular pathology.
Statistical Methods	**Sample size:** Approximately 16 patients from Study NS-065/NCNP-01-201 will enter this study. Because this is an open-label extension study conducted in patients who completed Study NS-065/NCNP-01-201, the sample size is not based on any statistical considerations. **Analysis Populations:** All analyses will be based on the actual treatment each patient received. Two populations will be defined for data analysis: the Safety Population and the Full Analysis Set (FAS). <u>Safety Population</u> All patients who receive at least one dose of NS-065/NCNP-01 Injection 250 mg in both the dose-finding (NS-065/NCNP-01-201) and extension study (NS-065/NCNP-01-202) will be included in the Safety Population. The Safety Population is the primary analysis population for safety assessments. <u>Full Analysis Set (FAS)</u> All patients who receive at least one dose of NS-065/NCNP-01 Injection 250 mg in both the dose-finding (NS-065/NCNP-01-201) and extension study (NS-065/NCNP-01-202) will be included in the FAS. The FAS is the

	primary analysis population for clinical efficacy and exploratory PD assessments. **General Statistical Considerations:** All measurements will be analyzed based upon the type of distribution and descriptive statistics presented by dose level and time point, as appropriate. Analyses utilizing pooled dose levels may also be performed. Interim analyses for safety are planned and interim analyses for efficacy may be conducted. **Efficacy Evaluation:** The primary efficacy outcome measure (TTSTAND), as well as secondary and exploratory outcome measures, will be summarized by dose level over time using descriptive statistics. Actual values and change from Pre-Infusion Visit (from Study NS-065/NCNP-01-201) and change from Week 25 (baseline of the NS-065/NCNP-01-202 study) will be presented. Outcomes will also be compared between NS-065/NCNP-01 Injection 250 mg and matched historical untreated controls possibly using a mixed-effects linear model. **Safety and Tolerability Evaluation:** Safety analyses will be performed using the Safety Population. Vital signs, 12-lead ECG, and clinical laboratory test results will be summarized by dose level over time using descriptive statistics. Actual values and change from Screening Visit (baseline of Study NS-065/NCNP-01-201) and change from Week 25 (baseline of Study NS-065/NCNP-01-202) will be presented. Treatment-emergent AEs (TEAEs) will be summarized by dose level, system organ class and preferred term (using the Medical Dictionary for Regulatory Activities (MedDRA) version 16.1), by dose level and relationship to study medication, and by dose level and intensity (CTCAE grade). All safety data will be listed.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

2'O-Me	2'O-methyl phosphorothioate
6MWT	Six minute walk test
aPTT	Activated partial thromboplastin time
ADL	Activities of Daily Living
AE	Adverse Event
ATC	Anatomical Therapeutic Chemical classification
AUC	Area under the curve
BMD	Becker muscular dystrophy
BNP	Brain Natriuretic Peptide
Cmax	Maximum Drug Concentration
Ca	Calcium
CGH	Comparative genomic hybridization
Cl	Chloride
CE	Clinical evaluator
CINRG	Cooperative International Neuromuscular Research Group
Cr	Creatinine
CS	Clinically significant
CK	Creatine kinase
Cm	Centimeter
CTCAE	Common Terminology Criteria for Adverse Events v4.03
CVA	Central Venous Access
CVC	Central Venous Catheter
CYP	Cytochrome P450
DMD	Duchenne muscular dystrophy

DNHS	Duchenne Natural History Study
DSMB	Data and Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic case report form
FDA	Food and Drug Administration
GC	Glucocorticoid
GCP	Good Clinical Practice
HEENT	Head, ears, eyes, nose, and throat
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed consent form
ICH	International Council of Harmonization
IND	Investigational New Drug
IP	Inorganic phosphorous
IPIM	Investigational Product Instruction Manual
IRB	Institutional Review Board
IV	Intravenous
K	Potassium
Kg	Kilogram
MCP-1	Monocyte chemoattractant protein-1
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified intention to treat
MLPA	Multiplex Ligation-dependent Probe Amplification
MM	Medical Monitor
mRNA	Messenger ribonucleic acid
MS	Mass spectrometry
MSO	Medical Safety Officer
MTD	Maximum tolerated dose
Na	Sodium
NAG	N-acetyl-beta-D-glucosaminidase
NCNP	National Center of Neurology and Psychiatry, Japan
NOAEL	No observed adverse effect level
NSAA	North Star Ambulatory Assessment
NSP	NS Pharma, Inc.
NS-065/NCNP-01	NS-065/NCNP-01 drug substance
NS-065/NCNP-01 Injection	NS-065/NCNP-01 drug product
NS-065/NCNP-01 Injection 250 mg	NS-065/NCNP-01 investigational drug product (vial strength)
PHI	Protected Health Information
PI	Principal Investigator
PD	Pharmacodynamic
PK	Pharmacokinetic
PMO	Phosphorodiamidate morpholino oligomer
PT-INR	Prothrombin – international normalization ratio
QMT	Quantitative Muscle Testing

QWBA	Quantitative Whole-Body Autoradiography
RT-PCR	Reverse transcriptase polymerase chain reaction
RBC	Red blood cell count
REB	Research Ethics Board
RIPA	Radioimmunoprecipitation assay
RNA	Ribonucleic acid
SAE	Serious Adverse Event
SAP	Statistical analysis plan
SD	Standard deviation
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SILAC	Stable isotope labeling using amino acids (in cell culture)
$t_{1/2}$	Terminal Elimination Half-Life
T_{max}	Time of Maximum Drug Concentration
TEAEs	Treatment-emergent AEs
TK	Toxicokinetic
TNF-α	Tumor necrosis factor-alpha
TTCLIMB	Time to Climb 4 stairs
TTRW	Time to Run/Walk 10 Meters
TTSTAND	Time to Stand
WBC	White blood cell count

1. INTRODUCTION

1.1. Duchenne Muscular Dystrophy– Epidemiology and Genetic/Biochemical Basis

Duchenne muscular dystrophy (DMD) is a disorder of progressive weakness leading to severe disability and ultimately death caused by a deficiency of the dystrophin protein. DMD is the most common form of muscular dystrophy, affecting 1 in every 3,500-6,000 live male births (1). The symptoms of DMD are often first noted at about 3-5 years of age, although clinical manifestations may be present as early as the first year of life. Proximal leg weakness impairs mobility and precludes the ability to run or to rise from a squatting position. Complete loss of ambulation follows, with a progressive decline of upper extremity strength and function. Declines in respiratory and cardiac function contribute to morbidity later in the disease, ultimately culminating in early lethality (1, 2). The impact of this debilitating condition on those affected by it and their families is significant.

The biochemical basis of DMD is the absence of a functional dystrophin protein that is essential for healthy muscle function and muscle fiber integrity. In normal striated muscle, the cytoplasmic dystrophin protein links intracellular actin with the extracellular matrix to provide structural stability of the muscle cell membrane. In the majority of patients with DMD, dystrophin protein is not produced because of out-of-frame mutations characterized by a deletion of one or more exons from the dystrophin gene, which is located on the short arm of the X chromosome. Dystrophin mutations in which some dystrophin protein function remains are associated with a similar, but often milder phenotype, classified as Becker muscular dystrophy (BMD). DMD and BMD exhibit X-linked recessive inheritance.

1.2. Current Natural History, Disease Management and Treatment Recommendations

The Cooperative International Neuromuscular Research Group (CINRG) (3) is conducting the largest prospective multicenter natural history study to date in DMD, the CINRG Duchenne Natural History Study (DNHS) (4, 5). The study includes >400 boys and men with DMD, followed for up to a decade at present. The study has annual follow-up visits. These visits include timed function tests, muscle strength, questionnaire functional assessments, pulmonary function tests and quality of life assessments. The DNHS database may be used as a reference dataset for the analysis of the cases in this study and Study NS-065/NCNP-01-201.

Since there is currently no cure for DMD, the goal of care is to provide the best quality of life through all stages of the disease. To date, treatments focus on optimizing strength and function through the use of pharmacological interventions, physical therapy and assistive and adaptive devices.

1.2.1. Glucocorticoid Treatment

At present, treatment with glucocorticoid (GC) medication is the only pharmacological intervention that has been shown to slow the decline of strength and function in DMD patients. The two main GCs used in DMD are prednisone and deflazacort (EMFLAZA™). Daily oral administration of prednisone or deflazacort stabilizes or improves strength and prolongs ambulation (6-11). The mechanism by which GCs are beneficial in dystrophin deficiency is likely multifactorial, including anti-inflammatory actions. The immunosuppressive effects of GCs may not be beneficial, and other immunosuppressants have not shown benefit (8).

In 2005, the American Academy of Neurology issued a practice parameter regarding GC treatment in DMD and recommended that GC should be offered as treatment, despite known side effects (12). The significant side effects of GCs include cushingoid features, adverse behavioral changes, obesity, growth retardation, increased risk for bone fractures, gastritis, delayed puberty, cataracts, hypertension, glucose intolerance, susceptibility to infection, and masking of response to stress (13). On February 9, 2017, the United States Food and Drug Administration (FDA) approved EMFLAZA™ to treat patients 5 years of age and older with DMD.

1.2.2. Dystrophin restoring interventions

New therapies based on specific genotypes are in development. Small molecules that can read through nonsense mutations could potentially treat approximately 10% of DMD patients (14). Exon skipping, which uses antisense oligonucleotides to alter the splicing pattern of the genes is designed to bring out-of-frame deletions into frame. The technology of exon skipping utilizes antisense oligonucleotides that bind to a specific sequence in the messenger ribonucleic acid (mRNA) to alter splicing of exons. By this means, specific exons can be excluded from the final transcript that is exported to the cytoplasm from the nucleus; hence the term ‘exon skipping’. By the design of the oligonucleotide, the out-of-frame deletion can be enlarged to include the adjacent exon such that the resulting deletion is in-frame (15-17).

This new type of treatment could potentially treat more than 85% of DMD patients who have large-scale deletion or duplication mutations in the dystrophin gene (15-17). The full characterization of DMD patient mutations and further development of the technology will be crucial to fully realize these novel therapies as they are developed.

To date, 2 oligonucleotide chemistries have been brought to the stage of human clinical trial: 2'O-methyl phosphorothioate (2'O-Me) antisense oligonucleotides (18, 19) and phosphorodiamidate morpholino oligomers (PMO) for skipping of exon 51 in the dystrophin gene (20-22).

A 48-week study of the 2'O-Me compound drisapersen did not reach significance in the six-minute walk test (6MWT), which was its primary outcome measure for a Phase II study (18). The PMO compound eteplirsen was tested in a 48-week study with the number of muscle fibers with restored dystrophin as its primary outcome measure (20). Eteplirsen (Exondys 51®) was approved by the FDA under the accelerated approval pathway on September 19, 2016.

1.3. Background on NS-065/NCNP-01

NS-065/NCNP-01 is a novel antisense oligonucleotide for the treatment of DMD, which has been discovered jointly by National Center of Neurology and Psychiatry (NCNP), which is a National Research and Development Agency in Japan, and Nippon Shinyaku Co., Ltd. Details of data summarized in the following sections can be found in the Investigator's Brochure.

1.3.1. Mechanism of Action

NS-065/NCNP-01 is designed to interact with the dystrophin gene ribonucleic acid (RNA), and alter the exon/intron splicing patterns. The mechanism of action is for NS-065/NCNP-01 to bind to a specific sequence in or near exon 53 of the dystrophin pre-RNA transcript, and block the exon/intron splicing of exon 53, leading to mature mRNA transcripts that lack exon 53. NS-065/NCNP-01 is thought to be effective on DMD patients with exon deletions amenable to skipping of exon 53 such as 43-52, 45-52, 47-52, 48-52, 49-52, 50-52, or 52. The loss of exon 53 restores the mRNA reading frame, thus converting a DMD (out-of-frame) deletion mutation to a Becker-like (in-frame) deletion mutation. In-frame deletion mutations are typically compatible with production of a shortened dystrophin protein, although the resulting Becker-like dystrophin protein will be smaller in molecular weight compared to the normal dystrophin protein, and likely

lower in abundance (quantity) compared to normal muscle, and thus may have lower function than normal amounts of wild-type dystrophin protein.

1.3.2. Summary of Non-Clinical Findings

In silico studies to identify potential off-target sequences suggest a very low probability that NS-065/NCNP-01, n-1, n-2 and shorter oligonucleotides would have an off-target effect, whereas n+1 oligonucleotides may have off-target effects on four genes (DHX16, DMRTA2 MALAT1, and CX3CL1) in humans.

NS-065/NCNP-01 did not display any adverse effects in *in vitro* and *in vivo* cardiovascular, *in vivo* CNS, or *in vivo* respiratory safety pharmacology studies.

1.3.3. Pharmacokinetics

Pharmacokinetic (PK) and toxicokinetic (TK) analyses revealed no apparent species differences for NS-065/NCNP-01. None of the *in vitro* or *in vivo* metabolism studies showed any distinct evidence of metabolism of NS-065/NCNP-01. After IV administration, T_{max} occurred at the first sampling time after the injection or at the end of the infusion for mice, rats, and monkeys. C_{max} and AUC increased with dose, and most increases were approximately proportional to dose, with some increases being greater than dose proportional. For rats, the mean values for $t_{1/2}$ were 1.19, 1.19, and 10.5 hours for 6, 20, and 60 mg/kg. For monkeys, the mean values for $t_{1/2}$ ranged from 1.7 to 3.5 hours. For mice and monkeys, exposure did not change with 12 or 13 weeks of repeat dosing.

Results for IM administration of NS-065/NCNP-01 to monkeys compared to IV administration indicate complete bioavailability, a longer time to T_{max}, a similar half-life for elimination, and no increase in exposure with repeat dosing.

The fraction of NS-065/NCNP-01 bound to rat, monkey, and human serum proteins was low, ≤40%, for all species and was independent of concentration. The distribution of NS-065/NCNP-01 into red blood cells was ≤2.5%, ≤6.7%, and ≤3.5% for rat, monkey, and human, respectively, indicating low distribution of NS-065/NCNP-01 to red blood cells. Quantitative whole-body autoradiography (QWBA) studies showed wide tissue distribution of [^{14}C]NS-065/NCNP-01 in both mice and monkeys, with the highest concentrations observed in the kidney, and general distribution to muscle tissues. For both rats and monkeys, renal excretion was the major route of

elimination, with less than 10% in the feces. No radioactivity was in the expired air from the rats. Most of the radioactivity was excreted within the first 24 hours. However, small measurable amounts continued to be excreted in the urine and feces throughout the seven-day collection periods.

The degree of inhibition of CYP1A2 by NS-065/NCNP-01 was low and the type of inhibition could not be determined. The inhibition was less than 30% for the other tested CYP isozymes: 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4/5. NS-065/NCNP-01 was not an inducer of CYP1A2, CYP2B6, or CYP3A4/5.

1.3.4. Toxicology

Repeated administration of NS-065/NCNP-01 via the clinically relevant route of administration (weekly IV injections) in both mice and monkeys resulted in decreases in red blood cell parameters, increased values for cytokines and histopathological effects in the kidney, testes and/or urinary bladder. The kidney is the primary target organ in both mice and monkeys, as shown by increased values in clinical chemistry parameters indicative of renal effects (1000 mg/kg/dose in 13- and 26-week mouse studies or 600 mg/kg/dose in a 12-week monkey study) and by histopathological findings of effects in renal tubules (≥240 mg/kg/dose in 13- and 26-week mouse studies, ≥200 mg/kg/dose in a 12-week monkey study or 360 mg/kg/dose in a 39-week monkey study) occasionally accompanied by increased kidney weight at necropsy. This observation is consistent with the PK data that show high tissue distribution to the kidney and excretion via the urine. An additional histopathological finding in a 26-week mouse study was the presence of cytoplasmic eosinophilic material in the transitional epithelium of the urinary bladder at 60 mg/kg/dose and above. At high dose (1000 mg/kg/dose in 13- and 26-week mouse studies or 600 mg/kg/dose in a 12-week monkey study), reductions in red blood cell parameters were also seen. Higher doses in mice (≥240 mg/kg/dose in a 13-week mouse study or 1000 mg/kg/dose in a 26-week mouse study) showed increased values for interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α) and/or monocyte chemoattractant protein-1 (MCP-1) and alterations in complement; these findings were considered to be due to irritation of the injection site. Data from 4- and 8-week recovery periods indicated a trend toward reversal of these effects in both species.

Moreover, no anti-NS-065/NCNP-01 antibodies were detected in the 13-week or 26-week mouse studies or in the 39-week monkey study. Anti-NS-065/NCNP-01 antibodies were detected in one monkey at 200mg/kg in the 12-week monkey study. However, antibody detection was not considered to affect the toxicological evaluation in this study, since skipping efficiency was confirmed in the muscle of this animal at the end of the treatment period and no remarkable decrease in exposure to NS-065/NCNP-01 was observed after repeated dosing. Based on these data, the NOAELs were concluded to be 60 and 15 mg/kg/dose in 13-week and 26-week mouse studies respectively and 60 mg/kg/dose in 12-week and 39-week monkey studies respectively. Both the in vitro and in vivo genotoxicity studies were negative.

NS-065/NCNP-01 was administered once weekly for 26 weeks at a dose level of 0 (vehicle: physiological saline), 50, 150 and 500 mg/kg (51 mice per group) male CByB6F1-Tg(HRAS)2Jic mice via intravenous route with a bolus injection. After the terminal necropsy, macroscopic examinations showed a mass and/or thickening in one side of the ureter in one mouse at 50 mg/kg, in two mice at 150 mg/kg, and no findings at 500 mg/kg dose group. In subsequent histopathological examinations on these three mice, transitional cell carcinoma was noted. Histopathological examination of the ureters for the other mice was conducted and no further tumorigenic changes were identified. Additionally, no treatment-related tumors were noted in any other organs. The blood concentration of NS-065/NCNP-01 in mice who received 50 mg/kg/wk was lower than the blood concentration of NS-065/NCNP-01 expected in human patients who will receive 80mg/kg/wk.

1.3.5. Summary of Clinical Findings

1.3.5.1. Phase I

A Phase I investigator-initiated study of NS-065/NCNP-01 Injection was conducted in DMD patients (aged 5-18 years) to investigate the overall usefulness of NS-065/NCNP-01 Injection in the treatment of DMD, based on evaluations of safety, exploration of predictive markers of treatment response, and assessment of pharmacokinetics. A total of 10 DMD patients were enrolled and randomized. IV administration of NS-065/NCNP-01 Injection in doses of 1.25 mg/kg, 5 mg/kg and 20 mg/kg to DMD patients once weekly for 12 weeks was well tolerated, and no

dose-limiting toxicity was observed, although all patients had at least one AE. Moreover, neither serious adverse events (SAE) nor incidences of Common Terminology Criteria for Adverse Events (CTCAE) v4.03 Grade 3 (severe) or worse were reported.

Among the mild and moderate AEs, an increase in urine beta-N-acetyl-D-glucosaminidase (Grade 1) was found in all patients in both cohorts 1 and 2 [Cohort 1 (n=3) Cohort 2 (n=3)] and in all but one patient in Cohort 3 [Cohort 3 (n=4)].

Initially, testing appeared to reveal proteinuria in 8 of 10 patients. However, it was subsequently determined that there was a cross reaction between NS-065/NCNP-01 and the pyrogallol red dye-binding method which was used for urinary protein measurement resulting in a false positive result for protein in the 24-hour pooled urine samples. To evaluate the 24-hour pooled urine samples for protein, Coomassie brilliant blue method was used to re-measure urinary protein in the frozen urine samples and none of the retested samples showed urinary protein levels exceeding the normal range of the institution (i.e., 31.2 to 120 mg/day). Current studies utilize a urine protein Benzethonium Chloride method using reagents that do not cross react with NS-065/NCNP-01. NS-065/NCNP-01 exhibited no cross reactivity with the Benzethonium Chloride Method up to 500 mg/dL.

The 24-hour pooled urine samples did not show increased levels of albumin. Spot measurements of urinary albumin were positive in 7 patients (Grade 1).

Interleukin levels were increased (Grade 1) in the serum in 6 out of 10 patients (high IL-6 level in 4 subjects, high IL-1β level in 1 subject, and high IL-2 level in 1 subject), and some level of anemia (Grade 1) was observed in 7 out of 10 patients.

Increased levels of brain natriuretic peptide (BNP) in serum (Grade 1) were present in four patients. White blood cell count was increased in 3 patients (Grade 1). All other mild and moderate AEs occurred in two or fewer patients.

The maximal plasma concentration and area under the curve $(AUC)_{0\text{-}t}$ values increased in a dose-dependent manner, and the $t_{1/2}$ value was between 1.52 and 1.84 hours.

Distinct exon 53 skipping efficiency by reverse transcriptase polymerase chain reaction (RT-PCR), positive dystrophin fibers by immunofluorescent staining, and dystrophin protein expression by

western blotting were detected in one patient in cohort 3, who was the largest patient enrolled in the cohort and hence received the largest absolute dose of NS-065/NCNP-01 Injection that was administered in the study.

1.3.5.2. Phase II Trials

NS065/NCNP01-P1/2 is a Phase I/II study of NS-065/NCNP-01 Injection being conducted in Japan. This is a multicenter, parallel-group, open-label, 24-week study. Participants receive weekly IV administration of NS-065/NCNP-01 Injection 250 mg (40 mg/kg and 80 mg/kg) over 24 weeks. The primary efficacy endpoint of this study is dystrophin protein expression as measured by Western blot, immunofluorescence staining, and RT-PCR. 16 DMD patients amenable to Exon 53 skipping, aged 5-<18 years are enrolled.

NS-065/NCNP-01-201 is a Phase II study conducted in the United States and Canada. This is a multicenter, 24-week dose finding study to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of NS-065/NCNP-01 in boys with DMD. Participants receive weekly IV administration of NS-065/NCNP-01 Injection 250mg (40 mg/kg and 80 mg/kg) or Placebo for the first 4 weeks, followed by weekly IV administration of NS-065/NCNP-01 Injection 250mg (40 mg/kg and 80 mg/kg) over the remaining 20 weeks. The primary efficacy endpoint of this study is dystrophin protein expression as measured by Western blot. 16 DMD patients amenable to Exon 53 skipping, aged 4-<10 years were enrolled.

1.4. Rationale for Study Design and Dose Selection

This is a Phase II, multicenter, open-label, extension study of NS-065/NCNP-01 Injection 250 mg administered intravenously once weekly for up to 192 weeks (up to a total of 216 weeks of treatment in Study NS-065/NCNP-01-201 and 202) or until enrollment in a separate long-term follow up program of NS-065/NCNP-01, whichever is earlier to boys with DMD who complete Study NS-065/NCNP-01-201. This study will evaluate the safety, tolerability, and clinical efficacy of NS-065/NCNP-01 Injection 250 mg at dose levels of up to 80 mg/kg/week, administered intravenously by infusion, over an additional treatment period of up to 192 weeks (up to a total of 216 weeks of treatment in Study NS-065/NCNP-01-201 and 202) or enrollment in a separate long-term follow up program of NS-065/NCNP-01, whichever is earlier. Patients who complete the Phase II Dose-finding Study NS-065/NCNP-01-201 are eligible to enroll at the same dose and will

undergo baseline assessments at Week 25 of Study NS-065/NCNP-01-201. In order to accommodate patient recovery following the scheduled biopsy during Week 25 of Study NS-065/NCNP-01-201, patients will be allowed a visit window of 7 days (±3 days) from the Week 24 dose to complete both the end of study procedures and the first infusion in the extension study. **All Week 25 study assessments, including muscle biopsy, of the NS-065/NCNP-01-201 study must be completed prior to first infusion in this extension study.** Patients can receive the first infusion of this extension study on the same day as the Week 25 study visit of the NS-065/NCNP-01-201 study, provided all study assessments (including muscle biopsy) of the NS-065/NCNP-01-201 study are completed prior to infusion.

Dose selection for Study NS-065/NCNP-01-201 was based on exposure data in humans compared to mice and monkeys over a comparable 12-week treatment period. Based on the $t_{1/2}$ values, the kinetics for monkeys are closer to human kinetics than the kinetics for mice. The AUC values for monkeys are higher than those for mice at similar doses. In the 12-week mouse study, the AUC_{0-24} values were 83.5, 421, and 12,300 µg•hr/mL for 60, 240, and 1,000 mg/kg, respectively. In the 12-week monkey study, the mean AUC_{0-24} values were 270, 963, and 5,581 µg•hr/mL for 60, 200, and 600 mg/kg, respectively. Hepatic and nuclease metabolism of NS-065/NCNP-01 was similar for monkey and human sources. Overall, there was no apparent degradation or metabolism of NS-065/NCNP-01 in the presence of hepatic microsomes, deoxyribonuclease I or phosphodiesterase I. Because monkeys are larger animals than mice, and because the half-life and metabolism is similar for humans and monkeys, the results of the monkey studies were chosen for the estimation of AUC values for human subjects.

Figure 1 shows the mean AUC_{0-t} values from the human clinical study and the mean AUC_{0-24} values from the single dose and 12-week monkey studies. The mean AUC values from both the monkey and human were approximately linear up to 200 mg/kg. At 200 mg/kg/day in Study TX1076, there were very slight effects on the kidneys of one of three male monkeys. The effects were more pronounced at 600 mg/kg/day, affecting three of three animals. There was little or no difference between Day 1 and Week 12 for either the humans or monkeys. The single-dose monkey study used doses that overlapped the doses used in the 12-week studies of humans and monkeys.

The individual AUC values for doses up to 200 mg/kg were subjected to regression analysis fitting the equation y = mx. The intercept was set to 0 since vehicle-treated animals had no measurable concentrations of NS-065/NCNP-01. The results for both Day 1 or single doses and after 12 weeks of dosing were included since there was no apparent difference between the results for the first and 12^{th} dose. Using the results for monkeys only, the coefficient of determination, r^2, was 0.9622, indicating a good fit to a line defined by AUC = 4,756 x Dose. The regression analysis was repeated using both the human and monkey results. The resulting equation was AUC = 4,760 x Dose with $r^2 = 0.9608$. The addition of the human results changed the slope by only 0.1%, and the fit remained good. The results are shown in Figure 2, and Figure 3 is an expansion showing the results between 0 and 80 mg/kg. At a dose of 60 mg/kg given to human subjects, the expected average AUC_{0-t} after either the first or multiple doses would be expected to be approximately 286,000 ng•hr/mL.

In the 12-week toxicity study using monkeys, the NOAEL dose was 60 mg/kg/dose, and very slight effects were seen at the 3.3-fold higher dose, 200 mg/kg/dose. A dose of 80 mg/kg is only 1.3-fold higher than a 60 mg/kg and less than ½ the 200 mg/kg dose where slight adverse effects were observed. It is possible that a human dose of 80 mg/kg producing an average AUC of 381,000 ng•hr/mL would have an acceptable safety profile.

Figure 1. Dose-Dependency of AUC_{0-t} or AUC_{0-24} for Humans and Monkeys

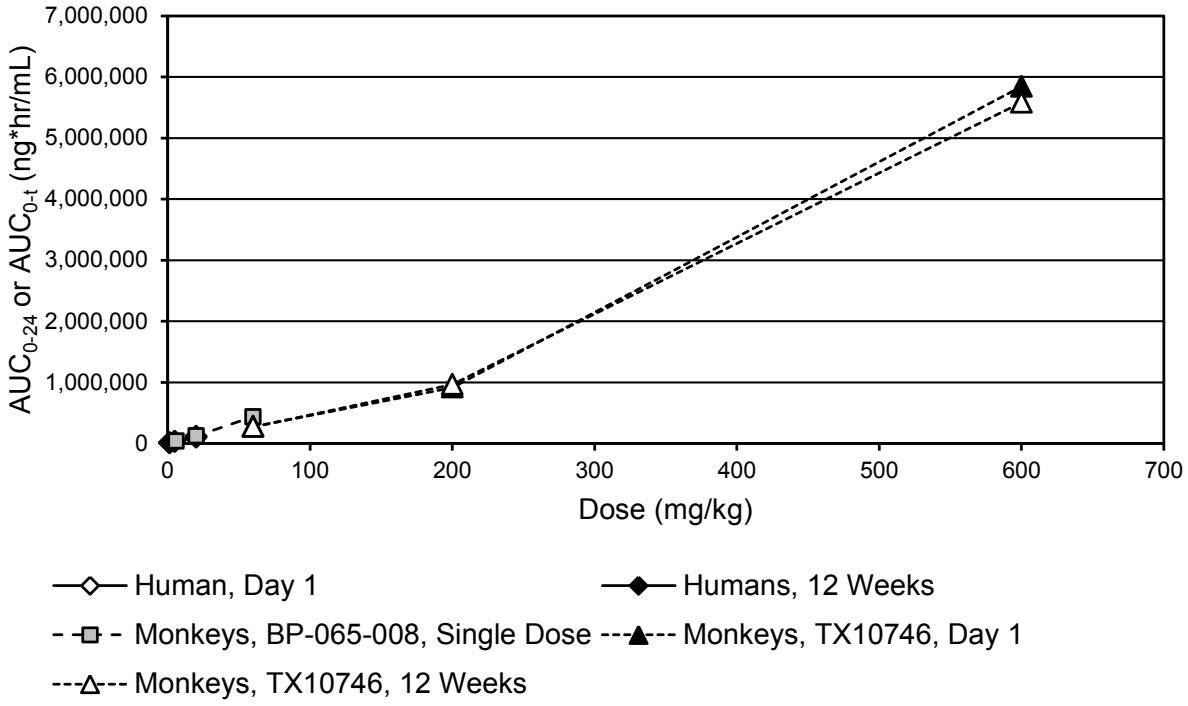


Figure 2. Correlation of AUC0-t or AUC0-24 with Dose from 0 to 200 mg/kg

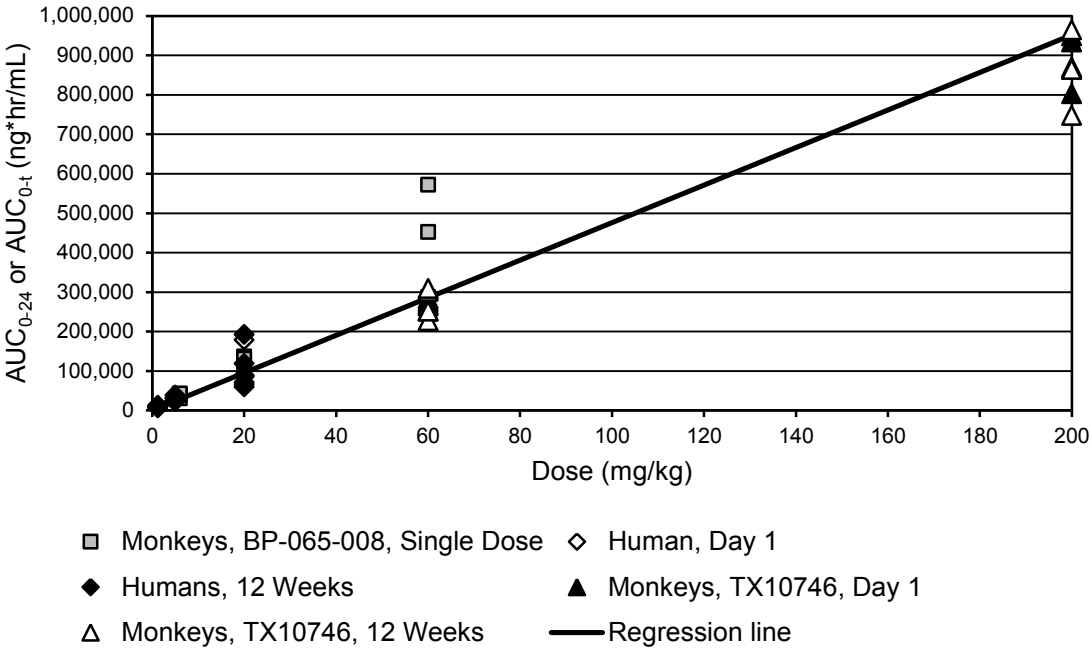
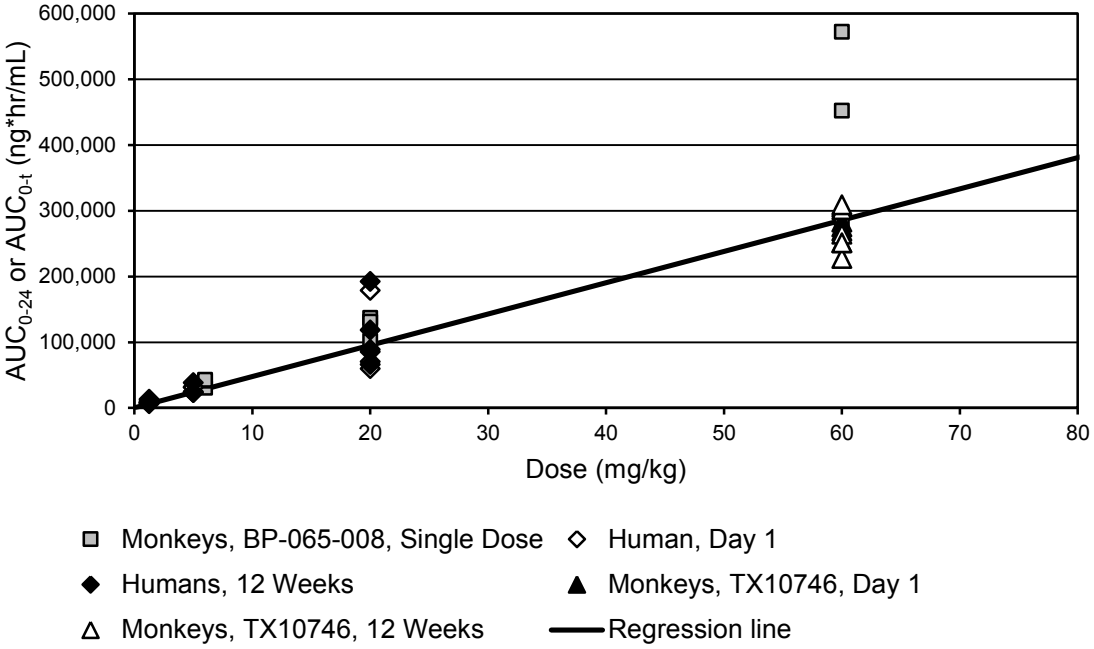


Figure 3. Expansion of Correlation from 0 to 80 mg/kg



AUC (ng•hr/mL) = 4,760 ((ng•hr/mL)/(mg/kg)) x Dose (mg/kg)
$r^2 = 0.9608$.

By the time of enrollment into the extension study NS-065/NCNP-01-202 the eligible patients are expected to have already successfully completed a 20-24-week treatment period at the 40 or 80 mg/kg/wk dosing level. Thus, the tolerability of the 2 dosing levels tested in study NS-065/NCNP-201 are no longer expected to be in question.

2. STUDY OBJECTIVES

2.1. Primary Objectives

1. To evaluate the safety and tolerability of low (40 mg/kg/week) and high (80 mg/kg/week) IV doses of NS-065/NCNP-01 Injection up to 192-week Treatment Period (up to a total of 216 weeks of treatment in Study NS-065/NCNP-01-201 and 202), in boys with DMD completing Study NS-065/NCNP-01-201.
2. To investigate the effect of low and high IV doses of NS-065/NCNP-01 Injection after an additional up to 192-week Treatment Period (up to a total of 216 weeks of treatment in Study NS-065/NCNP-01-201 and 202) on muscle strength, mobility, and functional exercise capacity, as measured by Time to Stand (TTSTAND), in boys with DMD who complete Study NS-065/NCNP-01-201 vs. matched historical controls in boys with DMD.

2.2. Secondary Objectives

1. To investigate the effect of low and high IV doses of NS-065/NCNP-01 Injection, after an additional up to 192-weeks treatment (up to a total of 216 weeks of treatment in Study NS-065/NCNP-01-201 and 202) on muscle strength, mobility, and functional exercise capacity, as measured by Time to Run/Walk 10 meters (TTRW), Time to Climb 4 stairs (TTCLIMB), North Star Ambulatory Assessment (NSAA), Six-minute Walk Test (6MWT), and Quantitative Muscle Testing (QMT) in boys with DMD that complete the 24-week dose-finding Study NS-065/NCNP-01-201 vs. matched historical controls in boys with DMD.

2.3. Exploratory Objectives

1. To investigate the effects of low and high IV doses of NS-065/NCNP-01 Injection on serum pharmacodynamic (PD) biomarkers.

3. INVESTIGATIONAL PLAN

3.1. Overall Study Design and Plan

This is a Phase II, open-label, extension study to assess the safety and efficacy of NS-065/NCNP-01 Injection in boys with DMD after completing the 24-week dose-finding study NS-065/NCNP-01-201.

Patients must complete all study assessments of the Week 25 of Study NS-065/NCNP-01-201 before performing any study assessments in this extension study. In this extension study (NS-065/NCNP-01-202) patients will continue to receive weekly study drug infusions at the dose level up to 80 mg/kg/week for up to 192 weeks (up to a total of 216 weeks of treatment in Study NS-065/NCNP-01-201 and 202) or enrollment in a separate long-term follow up program of NS-065/NCNP-01, whichever is earlier . Once sites receive IRB approval of Protocol Amendment 10, all subjects who were previously in the low dose cohort of 40 mg/kg/week will receive 80 mg/kg/week after obtaining patient consent/assent. Week 193 is a comprehensive assessment at the beginning of the extension visits incorporated in protocol amendment 10, and Week 217 is the final study assessment for the treatment period ending with Week 216. If the site is selected to participate in the separate long-term follow-up program and the patient agrees to participate by signing the consent form before the final study visit for the NS-065/NCNP-01-202 study, then the patient should complete the Early Termination visit and transition to the new study. The decision to transition to the follow up program will be made jointly by the patient/caregiver and investigator.

3.2. Design Implementation

3.2.1. Randomization

Study NS-065/NCNP-01-202 is an open-label extension study and not a randomized trial.

3.2.2. Investigational Product Dosing

The dose per patient (in mg) will be calculated based on the most recent body weight in kg collected per the protocol and not including the current visit. Details of dose preparation can be found in the Investigational Product Instruction Manual (IPIM). Doses will be administered by an IV infusion over approximately 1-hour. All missed or incomplete doses will be documented. The

dispensed study medication vials will be stored at the research site until drug accountability is verified by the pharmacy monitor.

Peripheral venous access is the preferred route of IV administration and should be used for all IP infusions throughout the study unless otherwise approved by the sponsor.

Implantable central venous access (CVA) ports will be considered on a case-by-case basis for participants who experience extreme difficulty with peripheral venous access. Discussions regarding implantable CVA ports for participants will include the site Principal Investigator (PI), Study Chair, Medical Monitor and Sponsor. Before final decision, NS Pharma will obtain documentation from the site investigator that the consulting surgeon who will place the port holds hospital privileges as a board eligible/board certified surgeon. Central venous access should not proceed without Sponsor approval.

An alternative method of central venous access may only be considered in the case of a contraindication, in the opinion of the consulting surgeon for the placement of a totally implantable central venous access device (port).

3.2.2.1. Low Dose Cohort

All patients in the low dose cohort of Study NS-065/NCNP-01-201 will continue to receive NS-065/NCNP-01 Injection at 40 mg/kg/week for up to 192-weeks (up to a total of 216 weeks of treatment in Study NS-065/NCNP-01-201 and 202) or enrollment in a separate long-term follow up program of viltolarsen, whichever is earlier . The PI can increase the dose from 40 mg/kg/week to 80 mg/kg/week once the site's IRB of record approves Protocol Amendment 10 and the patient signs the corresponding ICF. The first infusion of 80 mg/kg/week should be administered at the site.

3.2.2.2. High Dose Cohort

All patients in the high-dose cohort will continue to receive NS-065/NCNP-01 Injection at 80 mg/kg/week for up to 192-weeks (up to a total of 216 weeks of treatment in Study NS-065/NCNP-01-201 and 202) or enrollment in a separate long-term follow up program of NS-065/NCNP-01,

whichever is earlier.. If cumulative safety results do not warrant continuation at this dose level, the dose may be modified as described in Section 3.2.3.

3.2.3. Potential Design Modifications Due to Toxicities

Patients will receive the same dose in the extension study as they received in Study NS-065/NCNP-01-201 to ensure continuity of care and ongoing safety considerations for NS-065/NCNP-01 clinical data. Dose reductions may be necessary for individual patients. The dose level will be determined jointly by the Investigator, Study Chair and Medical Monitor in consultation with the Sponsor. Any modifications in the study design will occur in consultation with the Study Chair, Medical Monitor, Sponsor and the data and safety monitoring board (DSMB), depending on the timing and nature of the toxicities.

3.2.4 Dose Interruptions

Infusion interruptions may be necessary for individual participants. Infusion interruptions should be handled in accordance with standard procedures should an acute reaction occur during an infusion. Sites should notify the medical monitor in the event of an infusion interruption due to an acute reaction.

3.3. Study Duration

The expected study duration for each patient is approximately 192 weeks of treatment (up to a total of 216 weeks of treatment in Study NS-065/NCNP-01-201 and 202), plus a 30-day post-treatment phase, or until enrollment in a separate long-term follow up program of NS-065/NCNP-01, whichever is earlier. If the site is selected to participate in the separate long-term follow-up program, and the patient agrees to participate by signing the consent form before the final study visit for the NS-065/NCNP-01-202 study, then the patient should complete the Early Termination visit and transition to the new study. Week 193 is a comprehensive assessment at the beginning of the extension visits incorporated in protocol amendment 10, and Week 217 is the final study assessment for the treatment period ending with Week 216. The decision to transition to the follow up program will be made jointly by the patient/caregiver and investigator.

3.4 SARS-CoV-2 (COVID-19) Pandemic Study Impact

In early 2020, a global novel coronavirus outbreak of the SARS-CoV-2 (COVID-19) became a public health emergency. The full impact to this clinical trial is yet to be determined, however, provisions were put in place to ensure the safety of study participants as well as site staff. Memoranda were developed by the medical monitor which informed sites how to comply with protocol-specified procedures and mandated visits. While the COVID-19 pandemic is ongoing the 3rd and most recent of these memoranda may be updated on an ongoing basis to stay in alignment with FDA guidance related to the conduct of clinical trials during the COVID-19 pandemic. These documents are acknowledged by the IRBs of record as required. The full impact of COVID-19's impact on the clinical trial will be outlined in the final Clinical Study Report as well as the Statistical Analysis Plan.

4. STUDY POPULATION SELECTION

4.1. Study Population

Approximately 16 patients who complete the Phase II Dose-finding Study NS-065/NCNP-01-201 are eligible to enroll.

4.2. Inclusion Criteria

1. Patient's parent or legal guardian has provided written informed consent/HIPAA authorization prior to any extension study-specific procedures and patient has provided assent appropriate for his age and developmental status.
2. Patient completed Study NS-065/NCNP-01-201 through Week 25.
3. Patient and parent/guardian are willing and able to comply with scheduled visits, investigational product administration plan, and study procedures.
4. Patient must be on a stable dose of GC and is expected to remain on the stable dose of GC treatment for the duration of the study.

4.3. Exclusion Criteria

1. Patient had a serious or severe adverse event in Study NS-065/NCNP-01-201 that, in the opinion of the Investigator and/or the Sponsor, was probably or definitely related to NS-065/NCNP-01 Injection 250 mg use and precludes safe use of NS-065/NCNP-01 Injection 250 mg for the patient in this study.
2. Patient had a treatment which was made for the purpose of dystrophin, or dystrophin-related protein induction after completion of Study NS-065/NCNP-01-201.
3. Patient took any other investigational drugs after completion of Study NS-065/NCNP-01-201.
4. Patient is judged by the investigator and/or the Sponsor to not be appropriate to participate in the study for other reasons.

5. INVESTIGATIONAL PRODUCT

5.1. Description of NS-065/NCNP-01

Investigational product is provided in 10 mL or 5mL glass vials for dilution and IV administration.

- NS-065/NCNP-01: 10 mL or 5 mL glass vial containing 25 mg/mL or 50 mg/mL of drug substance solution in saline, respectively.

Description:

- NS-065/NCNP-01: Clear, colorless to pale yellow solution

Stability: NS-065/NCNP-01 Injection 250 mg is stable at 5 ± 3°C. Additional stability details can be found in the IPIM.

Storage conditions: Store refrigerated at 2° to 8°C.

Investigational product will be packaged, labeled and distributed to clinical sites and home-infusion central pharmacy by Xerimis in Moorestown, NJ. Additional details for ordering the investigational product can be found in the IPIM and study Manual of Operations.

5.2. Dispensing Investigational Product

Patients will begin dosing in this study at the same dose level they received in the Phase II NS-065/NCNP-01-201 dose-finding study. Patients will continue the dose level up to 80 mg/kg/week for the duration of up to 192-week (up to a total of 216 weeks of treatment in Study NS-065/NCNP-01-201 and 202)_study. Once sites receive IRB approval of Protocol Amendment 10, all subjects who were previously in the low dose cohort of 40 mg/kg/week will receive 80 mg/kg/week after obtaining patient consent/assent. Investigational product will be prepared in accordance with the IPIM and administered by IV infusion over approximately 1−hour.

5.3. Instructions for Administration of Investigational Product

Prepared investigational product (diluted solution) is administered intravenously within 6 hours of preparation and may be stored at room temperature during this time. Additional stability details can be found in the IPIM. A minimum of 3 days (72 hours) should elapse between treatments.

5.4. Blinding

Study NS-065/NCNP-01-202 is an open-label extension study and is not blinded.

5.5. Treatment Compliance

The patient's compliance with the treatment regimen will be monitored in terms of the patient receiving the investigational product infusion every week within a ±3-day window. Weekly study drug treatments for this study should be calculated from the 1st infusion of protocol NS-065/NCNP-01-201, not from the previous week's infusion. If an infusion day is rescheduled, the original scheme should be reinstated as soon as possible. Missed, delayed or incomplete infusions will be clearly documented and considered in the analysis. The amount of infusion received should be documented for all infusions.

5.6. Packaging and Labeling

Investigational product will be packaged and shipped from Xerimis directly to the investigative site and home infusion central pharmacy as a patient kit. Each patient kit consists of a single carton of 10 vials. Ancillary supplies will be provided by Xerimis with each patient kit. Investigational product will be labeled in compliance with 21CFR312.6, Labeling of and Investigational New Drug.

5.7. Storage and Accountability

Investigational Product Storage: Refrigerated (2-8°C), store in light resistant, airtight containers.

An identified, appropriate and secure storage location will be defined at each site's pharmacy and central home-infusion pharmacy for the investigational product. Additional details regarding proper handling of the investigative product can be found in the IPIM.

The investigator's or site's designated investigational product manager is required to maintain accurate investigational product accountability records. In the case of home-infusions, the central pharmacy staff is required to maintain accurate investigational product accountability records. All unused investigational product will be returned or disposed of as defined in the IPIM. This information will be included as part of the investigational product accountability record.

6. CONCOMITANT MEDICATIONS AND TREATMENTS

Lifetime use of GC and other pharmacological medications, including over the counter medications, herbal remedies, supplements and vitamins used after completion of the Week 25 of Study NS-065/NCNP-01-201 and throughout the study will be recorded in source documents and in the electronic case report form (eCRF). All medications taken throughout the study will be recorded in the eCRF. The following information will be collected: the medication name, dose, unit, frequency, route, indication, start and stop dates.

Any non-pharmacological treatment the patient has received after completion of the Week 25 of Study NS-065/NCNP-01-201 will be collected. The following information will be collected: name of treatment, indication, and start and stop date. Prior non-pharmacologic treatment will be recorded in source documents and captured in the relevant eCRF. Physical therapy schedule should not change and no new physical therapy should be started during the study. The need for changes to physical therapy should be discussed with the study chair prior to implementation.

6.1. Prohibited Medications

Concomitant medication use or changes to GC use are discouraged unless necessary for medical management. Any other experimental/investigational products are prohibited after completion of Study NS-065/NCNP-01-201 and throughout participation in this extension study. Patients who begin another investigational product will be withdrawn from the study.

6.2. Allowable Medications

Investigators may prescribe concomitant medications or treatments deemed necessary to provide adequate therapeutic and supportive care. Specifically, patients should receive full medical care during the study, including transfusions of blood and blood products, treatments with antibiotics, anti-emetics, anti-diarrheals, analgesics, topical or inhaled steroids, and other care as deemed appropriate, and in accordance with their institutional guidelines. All concomitant blood products, medications and supplements will be recorded in source documents and in the relevant eCRF.

7. STUDY PROCEDURES

7.1. Time and Events Schedule

The schedule of study assessments is described in Table 1; however, a patient can be seen at any time for reasons of safety. Study events are divided into the following phases:

- Informed Consent: Execution of the Informed Consent/HIPAA authorization/Assent will take place prior to or during the Week 24 study visit of Study NS-065/NCNP-01-201.
- Treatment Phase: The up to 192-week (up to a total of 216 weeks of treatment in Study NS-065/NCNP-01-201 and 202) interval of Study NS-065/NCNP-01-202, starting with administration of the first dose of study medication at Week 25 and continuing through the time of administration of the final dose of study medication. If the site is selected to participate in the separate long-term follow-up program and the patient agrees to participate by signing the consent form before the final study visit for the NS-065/NCNP-01-202 study, then the patient should complete the final study visit and transition to the new study. The decision to transition to the long-term follow up program will be made jointly by the patient/caregiver and investigator.
- Post-treatment Follow-up Phase: The up to 192-week Treatment Phase in study 202 (i.e. up to 216 weeks for the combined studies NS-065/NCNP-01-201 and 202) is followed by a final study visit with comprehensive safety and clinical efficacy assessments at the Week 217 visit. A 30-day follow-up interval begins after completion of the week 217 visit and ends after a final phone call for collection of any information about adverse events and concomitant medications for all participants. This 30-day follow-up interval and final phone call will only occur for patients who do not continue into a separate long-term follow up program of NS-065/NCNP-01. For subjects who do continue into the long-term follow up program, the Week 217 visit or the Early Termination (ET) visit will also serve as the baseline visit for the follow-up study.

  Subjects who terminate the 202 study before completing the 216 week treatment period will undergo an Early Termination (ET) visit which should include all safety and efficacy

assessments planned for the week 217 visit and they will complete the subsequent 30-day follow-up interval.

Table 1. Schedule of Study Assessments

Phase 2 Extension Study Protocol # NS-065/NCNP-01-202	NS-065/NCNP-201 Study [1]		Treatment Phase																												
Week	24	25	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
Window			±3 days window																												
Consent/Assent	X																														
Inclusion/Exclusion	X	X	X																												
Investigative Drug			**Investigative Drug**																												
Investigational Product Administration	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety Assessments			**Safety Assessments**																												
Weight		X					X				X				X				X				X				X				X
Height		X													X												X				
Vitals [2]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical and Neurological Exam		X					X				X				X				X				X				X				
Medical, Surgical, Medication, and Treatment History Review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hematology [3]		X					X				X				X				X				X				X				
Chemistry [4]		X					X				X				X				X				X				X				
BNP			X																								X				
Urinalysis (Random Urine) [5]		X					X				X				X				X				X				X				
Urinalysis (Pre-dose & Post-dose)																															
Urine (Cytology)[6]																															
Urinalysis (24hr pooled Urine)[7]	X																														
12-lead ECG		X													X												X				
Renal & Urinary Bladder Ultrasound[8]																															
Adverse Event review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cytokine [9]	X														X												X				
anti-dystrophin antibody	X														X												X				
anti-NS-065/NCNP-01 antibody	X														X												X				
Efficacy Assessments			**Efficacy Assessments**																												
Muscle biopsy (for dystrophin)		X																													
Function and Strength [10]		X													X												X				
Pharmacokinetics and Pharmacodynamics			**Pharmacokinetics and Pharmacodynamics**																												
PK (Blood)[11]	X														X												X				

Serum PD Biomarker			X													X											X				
Phase 2 Extension Study Protocol # NS-065/NCNP-01-202	Treatment Phase																															
Week	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86
Window	±3 days window																																
Consent/Assent																																	
Inclusion/Exclusion																																	
Investigative Drug	Investigative Drug																															
Investigational Product Administration	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety Assessments	Safety Assessments																															
Weight				X				X				X				X				X				X				X				X	
Height								X												X												X	
Vitals [2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical and Neurological Exam								X												X												X	
Medical, Surgical, Medication, and Treatment History Review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hematology [3]								X												X												X	
Chemistry [4]								X												X												X	
BNP																				X													
Urinalysis (Random Urine) [5]								X												X												X	
Urinalysis (Pre-dose & Post-dose)																																	
Urine (Cytology)[6]																																	
Urinalysis (24hr pooled Urine)[7]																																	
12-lead ECG																				X													
Renal and Urinary Bladder Ultrasound[8]																																	
Adverse Event review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cytokine [9]																				X													
anti-dystrophin antibody																				X													
anti-NS-065/NCNP-01 antibody																				X													
Efficacy Assessments	Efficacy Assessments																															
Muscle biopsy (for dystrophin)																																	
Function and Strength [10]								X												X												X	
PK and PD	Pharmacokinetics and Pharmacodynamics																															
PK (Blood)[11]																				X													

Serum PD Biomarker																			X												
Phase 2 Extension Study Protocol # NS-065/NCNP-01-202	**Treatment Phase**																														
Week	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116
Window	±3 days window																														
Consent/Assent																															
Inclusion/Exclusion																															
Investigative Drug	**Investigative Drug**																														
Investigational Product Administration	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety Assessments	**Safety Assessments**																														
Weight			X				X				X				X				X				X				X				
Height											X												X								
Vitals [2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical and Neurological Exam											X												X								
Medical, Surgical, Medication, and Treatment History Review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hematology [3]											X												X								
Chemistry [4]											X												X								
BNP											X																				
Urinalysis (Random Urine) [5]											X												X								
Urinalysis (Pre-dose & Post-dose)																															
Urine (Cytology)[6]																															
Urinalysis (24hr pooled Urine)[7]																															
12-lead ECG											X																				
Renal and Urinary Bladder Ultrasound[8]																															
Adverse Event review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cytokine [9]											X																				
anti-dystrophin antibody											X																				
anti-NS-065/NCNP-01 antibody											X																				
Efficacy Assessments	**Efficacy Assessments**																														
Muscle biopsy (for dystrophin)																															
Function and Strength [10]											X												X								
PK and PD	**Pharmacokinetics and Pharmacodynamics**																														
PK (Blood)[11]											X																				

Serum PD Biomarker										X																		

Phase 2 Extension Study Protocol # NS-065/NCNP-01-202	Treatment Phase																														
Week	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147
Window	±3 days window																														
Consent/Assent																															
Inclusion/Exclusion																															
Investigative Drug	**Investigative Drug**																														
Investigational Product Administration	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety Assessments	**Safety Assessments**																														
Weight	X				X				X				X				X				X				X				X		
Height					X												X												X		
Vitals [2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical and Neurological Exam					X												X												X		
Medical, Surgical, Medication, and Treatment History Review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hematology [3]					X												X												X		
Chemistry [4]					X												X												X		
BNP					X																								X		
Urinalysis (Random Urine) [5]					X												X												X		
Urine (Cytology)[6]																	X												X		
Urinalysis (24hr pooled Urine)[7]																															
12-lead ECG					X																								X		
Renal Ultrasound[8]																	X												X		
Adverse Event review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cytokine [9]																															
anti-dystrophin antibody					X																								X		
anti-NS-065/NCNP-01 antibody					X																								X		
Efficacy Asssessments	**Efficacy Asssessments**																														
Muscle biopsy (for dystrophin)																															
Function and Strength [10]					X												X												X		
Pharmacokinetics and Pharmacodyn	**Pharmacokinetics and Pharmacodynamics**																														
PK (Blood)[11]																															
Serum PD Biomarker					X																								X		

Phase 2 Extension Study Protocol # NS-065/NCNP-01-202	Treatment Phase																												
Week	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176
Window	±3 days window																												
Consent/Assent																													
Inclusion/Exclusion																													
Investigative Drug	**Investigative Drug**																												
Investigational Product Administration	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety Assessments	**Safety Assessments**																												
Weight		X				X				X				X				X				X				X			
Height										X												X							
Vitals [2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical and Neurological Exam										X												X							
Medical, Surgical, Medication, and Treatment History Review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hematology [3]										X												X							
Chemistry [4]										X												X							
BNP																						X							
Urinalysis (Random Urine) [5]										X												X							
Urine (Cytology)[6]										X												X							
Urinalysis (24hr pooled Urine)[7]																													
12-lead ECG																						X							
Renal Ultrasound[8]										X												X							
Adverse Event review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cytokine [9]																													
anti-dystrophin antibody																						X							
anti-NS-065/NCNP-01 antibody																						X							
Efficacy Asssessments	**Efficacy Asssessments**																												
Muscle biopsy (for dystrophin)																													
Function and Strength [10]										X												X							
Pharmacokinetics and Pharmacodyn	**Pharmacokinetics and Pharmacodynamics**																												
PK (Blood)[11]																													
Serum PD Biomarker																						X							

Phase 2 Extension Study Protocol # NS-065/NCNP-01-202	Treatment Phase																												
Week	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205
Window	±3 days window																												
Consent/Assent																													
Inclusion/Exclusion																													
Investigative Drug	**Investigative Drug**																												
Investigational Product Administration	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Safety Assessments	**Safety Assessments**																												
Weight	X				X				X				X				X				X				X				X
Height					X												X												X
Vitals [2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical and Neurological Exam					X												X												X
Medical, Surgical, Medication, and Treatment History Review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hematology [3]					X												X												X
Chemistry [4]					X												X												X
BNP																	X												
Urinalysis (Random Urine) [5]					X												X												X
Urine (Cytology)[6]					X												X												X
Urinalysis (24hr pooled Urine)[7]																													
12-lead ECG																	X												
Renal Ultrasound[8]					X												X												X
Adverse Event review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cytokine [9]																													
anti-dystrophin antibody																	X												
anti-NS-065/NCNP-01 antibody																	X												
Efficacy Asssessments	**Efficacy Asssessments**																												
Muscle biopsy (for dystrophin)																													
Function and Strength [10]					X												X												X
Pharmacokinetics and Pharmacody	**Pharmacokinetics and Pharmacodynamics**																												
PK (Blood)[11]																													
Serum PD Biomarker																	X												

Phase 2 Extension Study Protocol # NS-065/NCNP-01-202	Treatment Phase											Post-Treatment Phase	
Week	206	207	208	209	210	211	212	213	214	215	216	217 or ET	30-day Follow-up[13]
Window	±3 days window												
Consent/Assent													
Inclusion/Exclusion													
Investigative Drug	**Investigative Drug**												
Investigational Product Administration	X	X	X	X	X	X	X	X	X	X	X	X[12]	
Safety Assessments	**Safety Assessments**												
Weight				X				X				X	
Height												X	
Vitals [2]	X	X	X	X	X	X	X	X	X	X	X	X	
Physical and Neurological Exam												X	
Medical, Surgical, Medication, and Treatment History Review	X	X	X	X	X	X	X	X	X	X	X	X	X
Hematology [3]												X	
Chemistry [4]												X	
BNP												X	
Urinalysis (Random Urine) [5]												X	
Urine (Cytology)[6]												X	
Urinalysis (24hr pooled Urine)[7]													
12-lead ECG												X	
Renal Ultrasound[8]												X	
Adverse Event review	X	X	X	X	X	X	X	X	X	X	X	X	X
Cytokine [9]												X	
anti-dystrophin antibody												X	
anti-NS-065/NCNP-01 antibody												X	
Efficacy Asssessments	**Efficacy Asssessments**												
Muscle biopsy (for dystrophin)													
Function and Strength [10]												X	
Pharmacokinetics and Pharmacod	**Pharmacokinetics and Pharmacodynamics**												
PK (Blood)[11]													
Serum PD Biomarker												X	

Notes:

1. Week 25 of Study NS-065/NCNP-01-201 will also serve as Week 25 for first dose of Study NS-065/NCNP-01-202. End of study procedures of Study NS-065/NCNP-01-201 must be completed prior to dosing in Extension Study NS-065/NCNP-01-202
2. Perform vitals at pre-dose, at the end of infusion (+10 mins) and 1 hr (+/- 5 mins)
3. Hematology: RBC, Hb, Ht, Ret, MCV, MCH, MCHC, WBC, PLT, WBC Differential Fibrinogen, aPTT, PT-INR
4. Chemistry: Na, K, Cl, Ca, IP, BUN, Serum Creatinine, Cystatin C, AST, ALT, GGT, ALP, Haptoglobin, LDH/LDH isozyme, CK, Total bilirubin (Direct/Indirect), Total protein, Albumin, Albumin to globulin ratio, Total cholesterol, Triglyceride, Blood glucose, C-reactive protein
5. Urinalysis (Random Urine): Urine glucose, Urine blood, Urine urobilinogen, Urine specific gravity, Urine osmolarity, Urinary sediment (RBC, WBC, Cast), Urine protein (benzothonium chloride method), Albumin in urine, NAG in urine, α1-microgloblin in urine, Urine Creatnine
6. Urine Cytology is collected along with pre-dose Urinalysis. Following Protocol Amendment 9.0 approval, the assessment should be performed at the next clinic visit, then every 6 months thereafter (i.e. Visits 133, 157, 181 and 193 or Visits 145, 169, 193, 205 and 217). Urine Cytology is collected at Visit 217 for all subjects.
7. 24hr pooled Urine in Study NS-065/NCNP-01-201 only: Urine protein (benzothonium chloride method) , Albumin in urine, NAG in urine, α1-microgloblin in urine, Urine Cr, Urine Na, Urine K, Urine Cl, Urine IP, Uric acid in urine, β2-microgloblin in urine and PK
8. Renal Ultrasound includes imaging of kidneys, bladder and ureters. Following Protocol Amendment 9.0 approval, the assessment should be performed at the next clinic visit, then every 6 months thereafter (i.e. Visits 133, 157, 181 and 193 or Visits 145, 169, 193, 205 and 217). Renal Ultrasound is collected at Visit 217 for all subjects.
9. Cytokine: IL-6, TNF-α, MCP-1 at pre-dose and 8hr (±30min) after end of infusion
10. Function and Strength: Time to Stand (TTSTAND), Time to Climb (TTCLIMB), Time to run/walk 10 meters (TTRW), Six-minute walk Test (6MWT), North Start Ambulatory Assessment (NSAA), Quantitative Muscle Testing (QMT) and Ulnar Length
11. PK sampling timepoints: pre-dose, 1hr (+/- 5 mins) and 8hr (+/- 5 mins) after the end of the infusion
12. Only for subjects continuing in separate long-term follow-up study
13. Ony subjects who ET or do not continue in to separate long-term follow-up study

7.2. Informed Consent

Each patient's parent or legal guardian will receive an explanation of the nature and purposes of the study from the Investigator or designee. The Investigator or designee will ensure the study is appropriate for the patient. Consent must be obtained in accordance with the principles outlined in the current version of the Declaration of Helsinki. The patient's parent or guardian will confirm that s/he understands that the study is for research purposes only and that it may not provide any therapeutic benefit to the individual. Each patient's parent or guardian will confirm that s/he understands that the patient is free to withdraw from the study at any time without prejudice. The Investigator or designee will review the elements of the HIPAA and Protected Health Information (PHI) with each patient's parent or guardian and each patient's parent or guardian will confirm that s/he understands HIPAA authorization and PHI. The Investigator (or designated staff) will obtain the written informed consent and HIPAA authorization on the approved ICF by the appropriate IRB/REB at each site, from the patient's parent or guardian prior to any study-related procedures, including agreement to discontinuation of any prohibited medications, prior to the start of the study. The written assent of children will be obtained per individual site guidelines.

The ICF must be dated and signed by the Investigator or designee and the patient's legal representative and the original signed consent form must be kept by the Investigator in the study patient's file. "Legal representative" means an individual or judicial or other body authorized under applicable law to consent on behalf of a prospective study patient to the patient's participation in the procedure(s) involved in the research. The study patient's legal representative will receive a copy of the signed consent form.

If the ICF is amended during the study, the Investigator must follow all applicable regulatory requirements pertaining to all new patients and repeat the consent process with the amended ICF for any ongoing patients.

7.3. Assignment of Patient Identification Number

Study NS-065/NCNP-01-202 participation begins once written informed consent/Assent is obtained from the parent/legal guardian for a patient before any study-specific procedures are performed.

Following the signing of the written ICF/Assent Form, patients maintain their Study NS-065/NCNP-01-201 unique, site-specific, 6-digit patient identification number. All data will be identified using the unique patient identification number. The assigned patient identification number will be retained through enrollment and throughout participation in the study.

7.4. Demographics

Demographics will be collected during the Dose-finding Study NS-065/NCNP-01-201 and will remain in the EDC database for Extension Study NS-065/NCNP-01-202.

7.5. Medical History

Patient medical, surgical, medication and treatment history will be reviewed from Study NS-065/NCNP-01-201 and reviewed throughout the study. The dates and descriptions of past events will be documented in source documents and captured in the relevant eCRF.

7.6. Weight and Height

Height and weight will be collected at the visits specified in Table 1. Standing height will be collected with the patient barefoot (without shoes). The participant's legs should be kept as close together as possible and the heels should be placed back as close to the wall as possible. Participant may hold on to an object to facilitate balance. If a participant is non-ambulant, the ulnar length will be used to calculate height. Ulnar length will be measured by the CE during functional assessments (see Section 7.14.7). Weight will be collected with the patient barefoot and wearing light-weight clothes. Height and weight should take approximately 2 minutes and are not associated with any risks. These measurements are routinely performed during standard clinical examinations of patients with DMD. Weight in kilograms (kg) and height in centimeters (cm) will be documented in source documents and captured in the relevant eCRF.

7.7. Vital Signs

Vital signs will be performed at each study visit as specified in Table 1. For each visit that includes a study drug administration, vital signs will be performed at pre-dose, at the end of infusion (+10 mins) (approximately 1 hour after initiation of infusion), and 2 hours (+/- 5 mins) after initiation of infusion. If a clinically significant change from pre-dose is observed at 2 hours after initiation

of infusion, the parameter is followed every 30 mins (+/- 5 mins) until return to no clinical significant change from pre-dose.

Vital signs will include the following:

- Systolic blood pressure
- Diastolic blood pressure
- Heart rate
- Temperature

Vital signs will be documented in source documents and captured in the relevant eCRF. Any clinically significant changes noted by the investigator should be reported as an adverse event and should be addressed as clinically appropriate.

The site PI and the home infusion nurse will discuss vital sign parameters for each participant individually who is eligible and has elected to receive home infusions. To develop these guidelines, the site PI will take into consideration the range of clinically safe vital signs that have been observed from the participant during past study site infusions. The home infusion nurse will consult with the site investigator for any confirmed vital signs outside of these parameters (low or high).

7.8. Physical and Neurological Examination

The physical examination will be performed at the visits specified in Table 1 to assess any changes in physical presentation and symptoms. Physical examinations will include an assessment of the following:

- General appearance
- HEENT (head, ears, eyes, nose, and throat)
- Skin
- Lymph nodes
- Heart, including rhythm, heart sounds and presence of cardiac abnormalities
- Lungs
- Abdomen
- Extremities/joints
- Nervous system

- Any additional assessments necessary to establish baseline status or evaluate symptoms or adverse experiences

Documentation of the physical examination findings will be included in the source documentation at the clinical site. Only changes from baseline physical examination findings that meet the definition of an adverse event will be recorded on the adverse event page of the eCRF.

7.9. Renal and Urinary Bladder Ultrasound

Renal ultrasound will include imaging of the kidneys, ureters, and bladder and be performed at visits specified in the schedule of study assessments. Ultrasound of the kidneys, ureters and bladder will be assessed by local urologists or other trained medical professionals.

7.10. Adverse Events and Serious Adverse Events

Investigators will assess the occurrence of AEs and SAEs each study visit or patient contact during the study. AEs and SAEs may be reported by the patient/parent, discovered upon questioning, detected during examinations or review of test and lab results. AEs and SAEs should be documented in the source documents and the relevant eCRF with a full description including the nature, date and time of onset and resolution, determination of seriousness, severity, causality, corrective treatment, and outcome. Refer to Section 9 for safety procedures and reporting.

7.11. 12-Lead Electrocardiograms

A standard 12 lead ECG will be performed at the visits specified in the Schedule of Study Assessments (Table 1). ECG collection will be preceded by a 10-minute rest time during which the patient will remain in the supine position. At all time-points, ECGs will be collected prior to blood collection. ECG results will be based on machine readings and interpretation by local cardiologist or other trained medical professionals. ECG results will be recorded in the eCRF.

7.12. Clinical Laboratory Tests

Clinical laboratory assessments will be performed at visits specified in the Schedule of Assessments table (Table 1). Any blood sampling that occurs during the IP infusion should be collected from a location away from the IP infusion placement (i.e. opposite arm).

7.12.1. Sample Collection, Storage, and Shipping

Each patient will have blood drawn and urine collected for the blood and urine laboratory safety assessments as listed/described in the sections below, including hematology, chemistry, BNP, urinalysis, urine cytology, cytokines, anti-dystrophin antibody, and anti-NS-065/NCNP-01 antibody.

Laboratory safety assessments will be performed at the visits specified in Table 1. Samples will be collected by a trained member of the study team. This assessment is associated with the usual risks of a blood draw which include pain, bruise at the point where the blood is taken, redness and swelling of the vein, infection, and a rare risk of fainting. In order to decrease any of these possible risks the sites will employ pediatric trained staff and will use a numbing cream, if desired by the patient, to reduce the risk of pain.

All blood and urine samples will be sent to the designated central laboratory for testing unless otherwise noted. The procedures for the collection, handling, and shipping of laboratory samples will be specified in the Laboratory manual. Clinical laboratory tests are listed in **Error! Reference source not found.**.

Table 2. Clinical Laboratory Tests

Hematology, Chemistry, Urinalysis and Urine Cytology – Safety Labs

- Hematology
  - Red blood cell count
  - Hemoglobin
  - Hematocrit
  - Reticulocyte count
  - Mean corpuscular volume
  - Mean corpuscular hemoglobin
  - Mean corpuscular hemoglobin concentration
  - White blood cell count
  - White blood cell differential
  - Platelet count
  - Fibrinogen
  - Activated partial thromboplastin time
  - Prothrombin international normalization ratio

- **Blood Chemistry**
  - Sodium
  - Potassium
  - Chloride
  - Calcium
  - Inorganic Phosphorus
  - Blood Urea Nitrogen
  - Creatinine
  - Cystatin C
  - Aspartate aminotransferase
  - Alanine aminotransferase
  - Gamma-glutamyl transferase
  - Alkaline phosphatase
  - Haptoglobin
  - Lactate dehydrogenase
  - Creatine kinase
  - Total bilirubin (Direct/Indirect)
  - Total protein
  - Albumin
  - Albumin to globulin ratio
  - Total cholesterol
  - Triglyceride
  - Blood glucose
  - C-reactive protein

- **Brain Natriuretic Protein (BNP)**

- **Urinalysis**
  Urinalysis includes:
  - Urine glucose
  - Urine blood
  - Urine urobilinogen
  - Urine specific gravity
  - Urine osmolality
  - Urinary sediment (erythrocytes, WBC, Casts, epithelium, crystals)
  - Urine protein (benzethonium chloride method)
  - Urine protein (benzethonium chloride method)
  - Urine microalbumin
  - Urine N-acetyl-beta-D-glucosaminidase
  - Urine α1-microglobulin
  - Urine Creatinine
  - Urine Cytology

7.12.2. Cytokine Testing

Cytokine testing will be performed at visits as specified in the Schedule of Assessments (Table 1). The following tests will be performed: IL-6, TNF-α, MCP-1. Blood will be drawn from patients at pre-dose and 9 hours (+/- 30min) after initiation of infusion at Weeks 37, 49, 73, 97, and the early termination.

7.12.3. Anti-Dystrophin Antibody

Anti-dystrophin antibody testing will be performed on serum samples collected predose during the visits specified in the Schedule of Assessments (Table 1). Samples will be analyzed by Shin Nippon Biomedical Laboratories, Ltd. (Japan).

7.12.4. Anti-NS-065/NCNP-01 Antibody

Anti-NS-065/NCNP-01 antibody testing will be performed on serum samples collected pre-dose during the visits specified in the Schedule of Assessments (Table 1). Samples will be analyzed by Shin Nippon Biomedical Laboratories, Ltd. (Japan).

7.13. Pharmacodynamics and Efficacy Assessments

7.13.1. Serum Pharmacodynamic Biomarkers

Serum samples will be collected and stored for future pharmacodynamic biomarker studies.

7.14. Function and Strength

All function and strength testing will be performed by a trained clinical evaluator (CE). Assistive devices may be utilized during the testing as specified in the study Manual of Operations. The same CE should perform testing on the same patient throughout the study when possible.

7.14.1. Time to Stand (TTSTAND) - Primary Efficacy Endpoint

TTSTAND will be performed by a CE at visits specified in the schedule of study assessments (Table 1). This test will assess the time it takes the patient to go from lying flat on the floor to standing and is administered as part of the NSAA (see Section 7.14.4). This test should take approximately 1 minute and is not associated with any risks. This test is routinely performed during standard clinical examinations of patients with DMD. The number of seconds required to perform the test and the 6-point rating scale of how the patient attains the standing position will be documented in source documents and captured in the relevant eCRF.

7.14.2. Time to Run/Walk 10 Meters (TTRW)

TTRW will be performed by a CE at visits specified in the schedule of study assessments (Table 1). This test will assess the time it takes the patient to walk/run 10 meters including a 6-point rating scale for quality of the run/walk administered as part of the NSAA (see Section 7.14.4). This assessment should take 2 minutes and can be associated with falls; however, these are infrequently

reported. The number of seconds required to perform the test will be documented in source documents and captured in the relevant eCRF.

7.14.3. Time to climb 4 stairs (TTCLIMB)

TTCLIMB will be performed by a CE at visits specified in the schedule of study assessments (Table 1). This test will assess the time it takes the patient to climb 4 stairs (23) including a 6-point rating scale to assess how the patient negotiates the stairs administered as part of the NSAA (see Section 7.14.4). This test should take approximately 1 minute and is not associated with any risks. The number of seconds required to perform the test will be documented in source documents and captured in the relevant eCRF.

7.14.4. North Star Ambulatory Assessment (NSAA)

NSAA will be performed by a CE at visits specified in the schedule of study assessments (Table 1). The NSAA is a clinician rated, 17-item, functional scale originally designed for ambulant boys with DMD who are able to ambulate at least 10 meters (24). This evaluation tool assesses functional activities including standing, getting up from the floor, negotiating steps, hopping, and running. The assessment is based on a 3-point rating scale of 2 = ability to perform the test normally, 1 = modified method or assistance to perform test, 0 = unable to perform the test. Thus, total score can range from 0 (completely non-ambulant) to 34 no impairment on these assessments. Individual test item scores and total score will be recorded in source documents and in the relevant eCRF. This test should take approximately 10 minutes and is not associated with any risks. The NSAA should be administered before the 6MWT at each occurrence.

7.14.5. Six-minute Walk Test (6MWT)

6MWT will be performed by a CE at visits specified in the schedule of study assessments (Table 1). The 6MWT is a widely used and accepted test in numerous diseases. The version of the 6MWT adapted for use in DMD will be used (25). To perform the test, two points (cones) are set 25 meters apart and patients are asked to walk back and forth, between the cones quickly and safely for 6 minutes. The total distance in meters that the patient walks in 6 minutes is recorded. This test is considered a simple, standardized, low-technology and cost-effective means of clinically assessing 1) functional motor status and 2) integrated and global responses to exercise. The CE will measure the number of steps taken by the patient for the first 50 meters and total meters walked in 6 minutes. This test should take approximately 10 minutes. The 6MWT may cause feelings of

pressure or pain in the patient's chest, difficulty breathing, and shortness of breath. The 6MWT may also cause an increased risk of falling and muscle cramping.

7.14.6. Quantitative Muscle Testing (QMT)

QMT will be performed by a CE at visits specified in the schedule of study assessments (Table 1). QMT assessments are designed to measure muscle force production during an isometric contraction. QMT is a well-established method for measuring muscle weakness in neuromuscular disease (26). Patients will be placed on an examination table with a back-support system to eliminate the need for manual back stabilization. Following a single practice administration, each patient will complete a scored QMT evaluation (perform 2 tests; with the higher of the 2 values used for data analysis). QMT will be performed by recording force in pounds through a direct computer interface with a strain gauge. Testing positions and test order will be standardized. Bilateral testing of the muscle groups listed below will be performed:

- Handgrip
- Elbow flexors (biceps)
- Elbow extensors (triceps)
- Knee flexors (hamstrings)
- Knee extensors (quadriceps)

7.14.7. Ulnar Length

Ulnar length will be measured by a CE at visits specified in the schedule of study assessments (Table 1). In the event that a patient becomes non-ambulant the ulnar length will be utilized to impute height.

7.15. Pharmacokinetic Assessments

7.15.1. Collection and Assessment of Pharmacokinetic Samples

Blood samples for pharmacokinetic (PK) assessments will be taken at visits specified in the study assessment table (Table 1). PK sampling times are measured from the start of infusion. Infusion is expected to take approximately 1 hour to complete. PK samples will be analyzed in the event that anti-NS-065/NCNP-01 antibodies are detected in order to understand the impact of

immunogenicity on plasma levels of the drug. Blood will be drawn from patients at pre-dose, 2 hours (+/- 5 min) and 9 hours (+/- 5 min) after initiation of infusion at Weeks 37, 49, 73, and 97.

7.15.2. Shipment of Pharmacokinetic Samples

Plasma PK samples will be shipped frozen on dry ice according to instructions provided in the Laboratory Manual.

7.16. Total Blood Volume of Clinical Laboratory and Pharmacokinetic Samples

The total number of venipunctures and total volume of whole blood collected during the study will be limited to that needed for safety monitoring and efficacy assessments. Total scheduled whole blood volume collected over the study duration is not to exceed 321.1 mL for each patient. In the event of an SAE or an AE, additional blood may be taken for safety assessments based on timing and clinical judgement from PI, study chair and medical monitor. A breakdown of total volume of blood collected is summarized in **Error! Reference source not found.**.

Table 3. Blood Sample Number and Volume

Tests	WK 25[c]	WK 29	WK 33	WK 37	WK 41	WK 45	WK 49	WK 61	WK 73	WK 85	WK 97	WK 109	WK 121	WK 133	WK 145	WK 157	WK 169	WK 181	WK 193	WK 205	WK 217 or ET	Total mL of Blood
Safety	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	10.6	**222.6**
BNP	3.0						3.0		3.0		3.0		3.0		3.0		3.0		3.0		3.0	**27.0**
PD				1.0			1.0		1.0		1.0		1.0		1.0		1.0		1.0		1.0	**9.0**
PK[a,b]				6.0			6.0		6.0		6.0											**24.0**
Cytokine				4.0			4.0		4.0		4.0											**16.0**
Anti-dystrophin & Anti-NS-065/NCNP-01				2.5			2.5		2.5		2.5		2.5		2.5		2.5		2.5		2.5	**22.5**
Approximately total volume of blood per patient[e]																						**321.1**

a. Blood samples for PK analysis are collected according to the schedule in Section 7.15
b. For SAEs, additional blood draws for PK and immunogenicity will be based on timing and clinical judgement from PI, study chair and medical monitor.
c. Blood samples for safety analysis at Week 25 will be collected at the Week 25 of Study NS-065/NCNP-01-201.
d. Early termination (ET) blood draw volumes based on clinical judgement from PI, study chair and medical monitor.
e. Total volume of blood per patient does not include any additional testing that may be required for follow-up of AEs or re-testing and follow-up of abnormal laboratory results.
Total volume based on maximum expected blood draw from Week 25 through Week 217.

8. STUDY ACTIVITIES

8.1. Treatment Phase

In anticipation of the Week 50 study visit, which is the first study visit where optional home infusion could take place, the option of home infusion can be introduced to the patient by the study physician. Home infusion, which is limited to selected visits described below, is not required for participation in this study. The option of home infusion should be considered if the study physician believes that it will help the patient to derive safety, benefit and convenience from participation in the trial. The routine study visits that are eligible to be conducted in the home are limited to those whose study procedures do not extend beyond the following list: investigational product administration, vital signs collection, review of medical, surgical, medication and treatment history, and AE review. Specifically, visits 53, 57, 61, 65, 69, 73, 77, 81, 85, 89, 93, 97, 101, 105, 109, 113, 117, 121, 125, 129, 133, 137, 141, 145, 149, 153, 157, 161, 165, 169, 173, 177, 181, 185, 189, 193, 197, 201, 205, 209, 213 and 217 (or early termination) must be conducted at the study site and are not eligible for home infusion. Participation in optional home infusion requires written approval from the study sponsor, study chair, medical monitor and site PI. As advised by the study chair, medical monitor, and site PI, infusions taking place outside of the primary study site (e.g. home infusion) would only be considered starting at the Week 50 study visit or after, if the participant had not experienced any significant adverse events related to infusion of the investigational product. Patients must also reside in a municipality or region with access to emergency medical services (such as 911) to participate in home infusions. The study sponsor approval will only occur following endorsement by the Nippon Shinyaku Chief Medical Officer. The safety monitoring by the site PI, sponsor, study chair and medical monitor will continue throughout the treatment phase for all study visits, without regard for whether the infusion occurs at the study site or the home. Furthermore, the site PI is responsible for immediate contact with the study chair and medical monitor for any safety concern, without regard for whether the infusion occurs at the study site or the home.

All visit activities are to occur in the order in which they are listed below.

8.1.1. Day of First Infusion (Week 25)

The Week 25 study visit of Study NS-065/NCNP-01-201 shall also serve as the Week 25 dose visit if the patient wishes to continue into Extension Study NS-065/NCNP-01-202. In order to accommodate patient recovery following the scheduled biopsy during the Week 25 of Study NS-065/NCNP-01-201, patients will be allowed a visit window of 7 days (+/- 3 days) from the Week 24 infusion to complete both the end of study procedures and the first infusion in the extension study. All Week 25 study assessments of the NS-065/NCNP-01-201 study, including muscle biopsy, must be completed prior to first infusion in this extension study. Patients can receive the first infusion of this extension study on the same day as the Week 25 study visit of NS-065/NCNP-01-201, as long as all study assessments (including muscle biopsy) of NS-065/NCNP-01-201 are completed prior to infusion. Initial and Week 25 study assessments of NS-065/NCNP-01-201 will be used as baseline values for this extension study.

At Week 25 of Extension Study NS-065/NCNP-01-202, the following activities will occur:

- Inclusion/Exclusion Confirmation and Review
- Review of Medical, Surgical, Medication, and Treatment History (confirmation of concomitant medications and other treatments; any changes will be noted)*
- AE Review*
- Vital signs
- Blood draw for BNP (note this assessment may be collected with the other laboratory assessment draws for week 25 as part of Study NS-065/NCNP-01-201)
- Investigational product administration

*If Week 25 of the Extension Study occurs on the same day as Week 25 of NS-065/NCNP-01-201, these assessments do not need to be repeated.

8.1.2. Routine Infusion Visits

These visits will occur at: Weeks 26-28, 30-32, 34-36, 38-40, 42-44, 46-48, 50-52, 54-56, 58-60, 62-64, 66-68, 70-72, 74-76, 78-80, 82-84, 86-88, 90-92, 94-96, 98-100, 102-104, 106-108, 110-112, 114-116, 118-120, 122-124, 126-128, 130-132, 134-136, 138-140, 142-144, 146-148, 150-152, 154-156, 158-160, 162-164, 166-168, 170-172, 174-176, 178-180, 182-184, 186-188, 190-192, 194-196, 198-200, 202-204, 206-208, 210-212 and 214-216.

At these visits the following activities will occur:

- Review of Medical, Surgical, Medication and Treatment History
- AE Review
- Vital Signs
- Investigational product administration

8.1.3. Weeks 29, 33, 41, and 45

At these visits the following activities will occur:

- Review of Medical, Surgical, Medication, and Treatment History
- AE Review
- Weight
- Vital Signs
- Physical and Neurological Exam
- Hematology
- Chemistry
- Urinalysis (Random Urine)
- Investigational product administration

8.1.4. Week 37, 49, 73, and 97

At the visit the following activities will occur:

- Review of Medical, Surgical, Medication, and Treatment History
- AE Review
- Height & Weight
- Vital Signs
- Physical and Neurological Exam
- 12-Lead ECG
- Function and Strength:
    - TTSTAND
    - TTRW
    - TTCLIMB

- NSAA
- 6MWT
- QMT
- Ulnar Length

- Hematology
- Chemistry
- Blood draw for BNP (at Week 49, 73, and 97)
- Urinalysis (Random Urine)
- Cytokine
- Anti-dystrophin antibody (pre-dose)
- Anti-NS-065/NCNP-01 antibody (pre-dose)
- Serum PD biomarker
- Investigational product administration
- PK Blood

8.1.5. Routine Infusion Visits with Weight Collection

These visits occur at: Weeks 53, 57, 65, 69, 77, 81, 89, 93, 101, 105, 113, 117, 125, 129, 137, 141, 149, 153, 161, 165, 173, 177, 185, 189, 197, 201, 209, and 213. At these visits the following activities will occur:

- Review of Medical, Surgical, Medication, and Treatment History
- AE Review
- Weight
- Vital Signs
- Investigational product administration

8.1.6. Weeks 61, 85, 109, 133, 157, 181, and 205

At these visits the following activities will occur:

- Review of Medical, Surgical, Medication, and Treatment History
- AE Review
- Height & Weight,

- Vital Signs
- Physical and Neurological Exam
- Function and Strength:
    - TTSTAND
    - TTRW
    - TTCLIMB
    - NSAA
    - 6MWT
    - QMT
    - Ulnar Length
- Hematology
- Chemistry
- Urinalysis (predose and first void taken within 5 hours postdose)
- Urine Cytology (pre-dose) (133, 157 and 181 only)
- Renal and Urinary Bladder Ultrasound (Visits 133, 157 and 181 only)
- Investigational product administration

8.1.7. Week 121, 145, 169, and 193

At the visit the following activities will occur:

- Review of Medical, Surgical, Medication, and Treatment History
- AE Review
- Height & Weight
- Vital Signs
- Physical and Neurological Exam
- 12-Lead ECG
- Function and Strength:
    - TTSTAND
    - TTRW
    - TTCLIMB
    - NSAA

- 6MWT
- QMT
- Ulnar Length

- Hematology
- Chemistry
- Blood draw for BNP
- Urinalysis (predose and first void taken within 5 hours postdose)
- Urine Cytology (pre-dose) (Visits 145 and 169 only)
- Renal and Urinary Bladder Ultrasound (Visits 145 and 169 only)
- Anti-dystrophin antibody (pre-dose)
- Anti-NS-065/NCNP-01 antibody (pre-dose)
- Serum PD biomarker
- Investigational product administration

8.2. Post-Treatment Phase

8.2.1. Week 217 or Early Termination Visit

The following activities will occur at the end of the treatment (Week 217) for all patients. If a patient withdraws from the study or is withdrawn by the site PI (Early Termination Visit) the activities listed below should be completed. All visit activities are to occur in the order in which they are listed below.

- Review of Medical, Surgical, Medication, and Treatment History
- AE Review
- Height & Weight
- Vital Signs
- Physical and Neurological Exam
- 12-Lead ECG
- Function and Strength:
  - TTSTAND
  - TTRW

  - TTCLIMB
  - NSAA
  - 6MWT
  - QMT
  - Ulnar Length
- Hematology
- Chemistry
- Blood draw for BNP
- Urinalysis (Random Urine)
- Urine Cytology
- Renal and Urinary Bladder Ultrasound
- Serum PD biomarker

8.2.2. Follow-Up Phone Call

Participants who do not enroll in a separate long-term follow up program of NS-065/NCNP-01 will have a phone call with a member of the site study staff, 30 days (+3 days) following Week 217/ET visit, to assess adverse events and document changes in concomitant medications. Any AEs that are unresolved at the patient's last AE assessment in the study are followed up by the site PI or designee for as long as medically indicated, but without further recording in the eCRF. The CRO retains the right to request additional information for any patient with ongoing AEs at the end of the study, if judged necessary.

8.2.4. Early Termination or Withdrawal from the Study

A patient (or the legal guardian acting on behalf of the patient) is free to withdraw consent and discontinue participation in the study at any time, without prejudice to further treatment according to standard clinical practice. Study participation may be discontinued at any time at the discretion of the site PI or Sponsor. The following may be justifiable reasons for removing a patient:

- Withdrawal of consent by the patient/legal guardian;
- Failure to comply with the protocol;
- Lost-to-follow-up;

- Illness, condition, or procedural complication (including adverse events) affecting the patient's ability to participate or requiring prohibited medication;
- In the Investigator's judgment, it is deemed in the best interest of the patient to discontinue his/her participation in the study;
- The study drug becomes commercially available
- The Investigator, Sponsor, DSMB and/or regulatory authority terminates the study; or
- Any other reason.

A Patient Completion/Discontinuation eCRF describing the reason for discontinuation must be completed, for any discontinued or withdrawn patient regardless of reason. If a patient withdraws from the study or if the study is prematurely terminated, the site PI or designee will contact the patient or the patient's legal guardian **within 30** days after withdrawal or termination to assess any AEs. The site PI will be asked to follow all SAEs until the event returns to baseline or until the site PI determines that follow-up is no longer medically necessary. Patients who are withdrawn from the study may not re-enter.

If a patient is lost to follow-up, every reasonable effort must be made by the clinical site personnel to contact the patient and determine the reason for discontinuation/withdrawal (including assessment of any AEs reported by the patient/caregiver). The measures taken to follow-up must be documented in source documents.

8.2.5. Procedures for Early Termination

If a patient withdraws or is removed from the study for any reason, all early termination procedures should be completed. Reason for withdrawal, date of the discontinuation, and date of the last dose of investigational product should be recorded in source documents and in the appropriate section of the eCRF. Investigational product assigned to the withdrawn patient may not be assigned to another patient.

The Medical Monitor and Study Chair should be consulted prior to the withdrawal of the study patient, except in the case of a medical emergency. Written notice (regardless of cause) is to be provided to the Medical Monitor within 48 hours of the withdrawal. At the time of discontinuation, every effort should be made to ensure all relevant procedures and evaluations scheduled for the

final study visit are performed. Except in the case of a medical emergency, assessments described in Section 8.2.1, Week 217 or Early Termination Visit, will be performed.

8.3. Unscheduled Visit

If a patient returns to the clinic for a visit outside of the protocol evaluation time points, the visit and any assessments and/or tests performed will be recorded into the source documents and the eCRF as an Unscheduled Visit.

8.4. Patient Replacement

If a patient withdraws during the extension study, he will not be replaced.

8.5. Suspension or Termination of Study

If, in the opinion of the Study Chair, and the Medical Monitor, clinical observations in the study suggest that it may be unwise to continue, the study may be suspended. The Study Chair will request a DSMB meeting and consult with the Sponsor. If the Study Chair, Medical Monitor, DSMB and Sponsor agree that safety concerns warrant termination of the study, the Sponsor will terminate the study. A written statement fully documenting the reasons for such a termination will be provided to investigators, IRBs/REBs and regulatory authorities, if required.

NS Pharma has the right to terminate an Investigator's participation in the study and remove all study materials from a clinical site. A written statement will be provided to the Investigator, the IRB/REB, and regulatory authorities, if required.

Possible reasons for termination of the study at a clinical site include, but are not limited to:

- Unsatisfactory enrollment with respect to quantity or quality,
- Inaccurate or incomplete data collection on an ongoing basis,
- Falsification of records, or
- Failure to adhere to the protocol.

If any serious or non-serious adverse events have occurred at such a clinical site, all documentation relating to the event(s) must be obtained.

9. SAFETY PROCEDURES AND PROCESSES

9.1. Definition of an Adverse Event

An AE is any untoward medical occurrence in a patient or clinical investigation patient administered a pharmaceutical product, including control, and which does not necessarily have a causal relationship with treatment. This includes any untoward signs or symptoms experienced by the patient from the time of consent until completion of the study.

AEs may include, but are not limited to:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.
- Any new disease or exacerbation of an existing disease.
- Any deterioration in non-protocol-required measurements of laboratory value or other clinical test (e.g., ECG) that results in symptoms, a change in treatment, or discontinuation from investigational product.

Disease signs, symptoms, and/or laboratory abnormalities already existing prior to the use of the product are not considered AEs after treatment, unless they reoccur after the patient has recovered from the preexisting condition or in the opinion of the Investigator they represent a clinically significant exacerbation in intensity or frequency. If clinically significant worsening from baseline is noted, the changes will be documented in the AE source document and the eCRF.

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

Suspected adverse reaction means any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of IND safety reporting, ‘reasonable possibility’ means there is evidence to suggest a causal relationship between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been previously observed. During the course of the study, the Investigator's Brochure should be updated on an ongoing basis with new important safety information.

9.2. Definition of a Serious Adverse Event

An adverse event is serious when the patient outcome is:

- Death
- Life-threatening (see below for expanded definition)
- Hospitalization (initial or prolonged)
- Disability or permanent damage (see below for expanded definition)
- Congenital anomaly/Birth defect
- Important medical events that, based upon appropriate medical judgment, may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

Life-threatening Experience: Any AE that places the patient, in the view of the site PI, at immediate risk of death from the AE as it occurred, i.e. does not include an AE that, had it occurred in a more severe form, might have caused death.

Any hospital admission with at least one overnight stay will be considered an inpatient hospitalization. However, emergency room visits that do not result in admission to the hospital should be evaluated for one of the other serious outcomes (e.g., life-threatening; required intervention to prevent permanent impairment or damage; other serious medically important event).

Hospitalization for an elective or outpatient procedure scheduled or planned before signing of informed consent will not be considered to be an SAE. However, unexpected complications and/or prolongation of hospitalization that occur during elective or outpatient surgery should be recorded as adverse events and assessed for seriousness. Admission to the hospital for social or situational reasons (e.g., no place to stay, live too far away to come for hospital visits) will not be considered inpatient hospitalizations.

Disability or permanent damage: Any AE that results in a substantial disruption of a patient's ability to conduct normal life functions, i.e., the AE resulted in a significant, persistent or permanent change, impairment, damage or disruption in the patient's body function/structure, physical activities and/or quality of life.

Important medical events that may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above: an AE that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

9.3. Severity

It is the Investigator's responsibility to assess the intensity (severity) of an adverse event.

The severity of the adverse event will be characterized and recorded as "mild, moderate, severe, life-threatening or death" according to the following definitions: The CTCAE v4.03 guidelines for severity assessments will be used to grade adverse events for this trial (available at evs.nci.nih.gov/ftp1/CTCAE/About.html). The CTCAE v4.03 listed guidelines for severity assessment are:

- Mild: Asymptomatic or mild symptoms; clinical or diagnostic observations only or intervention not indicated
- Moderate: minimal, local or noninvasive intervention indicated or limited age-appropriate instrumental activities of daily living (ADL)
- Severe: Severe or medically significant but not immediately life-threatening; or hospitalization or prolongation of hospitalization indicated; or disabling; or limiting self-care ADL
- Life-threatening: life-threatening consequences or urgent intervention indicated
- Death: Death related to AE

Note: A severe adverse event need not be serious and a serious adverse event (SAE) need not, by definition, be severe.

9.4. Relationship

It is the Investigator's responsibility to assess the relationship between the investigational product and the adverse event. The degree of "relatedness" of the adverse event to the investigational product may be described using the following scale:

Not Related

- **Not Related**: No temporal association and other etiologies are likely the cause.
- **Unlikely**: Event or laboratory test abnormality, with a time to drug that makes a relationship improbable (but not impossible). Diseases or other drugs provide plausible explanations.

Related

- **Possible**: Temporal association, but other etiologies are likely the cause. However, involvement of the investigational product cannot be excluded.
- **Probable**: Temporal association, other etiologies are possible but unlikely. The event may respond if the investigational product is discontinued.
- **Definite**: Established temporal association with administration of the investigational product with no other more probable cause. The event should resolve when the investigational product is discontinued and recur on re-challenge.

9.5. Reporting

9.5.1. Adverse Event Reporting

All adverse events occurring during the course of the study (starting from signing informed consent to study completion) will be collected on the adverse event eCRF. Each adverse event is to be evaluated for duration, severity, seriousness, and causal relationship to the investigational product. For each adverse event, the following information will be recorded:

- Description of the event (e.g., headache),
- Date of onset,
- Date of resolution (or that the event is continuing),
- Action taken as a result of the event,
- Seriousness of the event,

- Severity of the event,
- Outcome of the event, and
- Investigator's assessment of relationship to investigational product.

A cluster of signs and symptoms that results from a single cause should be reported as a single adverse event (e.g., fever, elevated WBC, cough, abnormal chest x-ray, etc., can all be reported as "pneumonia").

The Investigator will carefully evaluate the comments of the patient and the response to treatment in order that he/she may judge the true nature and severity of the adverse event. The question of the relationship of adverse events to investigational product administration should be determined by the Investigator or study physician after thorough consideration of all facts that are available.

Clinically significant (CS) changes from time of informed consent will be documented as AEs on the AE eCRF. CS changes are physical findings that have medical relevance and may result in an alteration in medical care.

9.5.2. Serious Adverse Event Reporting

All SAEs, including death due to any cause and whether or not deemed drug-related or expected, must be reported within the study EDC system (eCRF) within 24 hours (1 working day) of the Principal Investigator or the clinical site becoming aware of the occurrence.

The Investigators responsible for ongoing clinical studies with the investigational product will be notified by the CRO of all SAEs.

▪ 9.5.3 Adverse Events of Special Interest

The following events are considered AEs of special interest based on the route of administration and toxicology profile for the study drug:

- Access device complication (for participants with indwelling access devices);
- Cystatin C >1.5 × ULN or >1.5 × baseline if baseline >ULN;
- Urine protein-osmolality > 0.3 mg/L/mOsm/kg;
- Any confirmed instances of hematuria or other potentially clinically significant abnormalities on urinalysis.

9.6.Serious Adverse Event Follow-up

SAEs will be followed by the site investigator until resolution or until the site PI determines that follow-up is no longer medically necessary.

Follow-up information, or new information regarding an ongoing SAE, must be provided promptly to the CRO within 24 hours of knowledge of the new or follow-up information. The Coordinating Center will forward the information to the Study Chair, Sponsor, the Medical Monitor and DSMB.

9.7. Reporting of Serious Adverse Events to Regulatory Authorities

PharmaLex is responsible for submitting Serious and Unexpected Suspected Adverse Reaction (SUSAR) reports of SAEs to the appropriate regulatory authorities. All Investigators responsible for ongoing clinical studies with the investigational product will be notified by the CRO of all SAEs. The investigator shall report all SAEs occurring within 24 hours after becoming aware via the study EDC system. In the event the EDC system is down, the SAE report form should be completed and emailed or faxed to IQVIA. Reports of all SAEs must be communicated as soon as possible to the appropriate IRB/REB and/or reported in accordance with local laws and regulations. Investigators should provide written documentation of IRB/REB notification for each report to CRO.

The sponsor must report any suspected adverse reaction to the study medication, that is both serious and unexpected, or any serious adverse events suspected to be related to the CVA port, to the FDA and Health Canada (21 CFR 312.32(c)(1)(i) and C.05.014, respectively).

9.8. Reporting of Patient Death

The death of any patient during the study or within 30 days of study completion, regardless of the cause, must be reported as detailed in Section 9.5.

9.9. Monitoring and Follow-up of Adverse Events

Patients who experience adverse events will be monitored with relevant clinical assessments and laboratory tests, as determined by the Investigator. All follow-up results are to be reported to the Medical Monitor. Any actions taken and follow-up results must be recorded either on the appropriate page of the eCRF- or in appropriate follow-up written correspondence, as well as in

the patient's source documentation. Follow-up laboratory results should be filed with the patient's source documentation.

For all adverse events that require the patient to be discontinued from the study, relevant clinical assessments and laboratory tests must be repeated at appropriate intervals until final resolution or stabilization of the event(s).

Any AEs that are unresolved at the patient's last AE assessment in the study are followed up by the site PI or designee for as long as medically indicated, but without further recording in the eCRF. The sponsor and designated CRO retain the right to request additional information for any patient with ongoing AEs/SAEs at the end of the study, if judged necessary.

9.10. General Monitoring and Management of Abnormal Clinical Labs

It is the Investigator's responsibility to review the results of all laboratory tests as they become available and to sign and date the results indicating review. For each laboratory test outside of the laboratory normal range, the Investigator must ascertain if this represents a clinically significant change from baseline for the individual patient. The Investigator may repeat a laboratory test or request additional tests to verify results of the original laboratory test.

If a laboratory value is determined to be an abnormal and clinically significant change from baseline for the patient, the Investigator should determine if it qualifies as an adverse event, and if so, an appropriate eCRF will be completed. All clinically significant laboratory abnormalities occurring during the study and that were not present at baseline should be followed and evaluated with additional tests if necessary until diagnosis of the underlying cause, or resolution.

9.11 Monitoring of Urine Analyses

Any instances of hematuria or other potentially clinically significant abnormalities on urinalysis will be confirmed at the following week's visit, or sooner at discretion of the investigator. Confirmed clinically significant treatment-emergent abnormalities will be recorded as AEs and discussed with the study chair and medical monitor. Abnormalities will be monitored and evaluated with additional tests or consultations, if necessary, until the underlying cause is determined, or the event is brought to an acceptable resolution. Additional clinical and laboratory

information will be collected and documented in order to better characterize abnormalities and identify etiology and appropriate management.

9.12. Monitoring and Management of Abnormal Electrocardiograms

If a clinically significant ECG abnormality occurs that was not present at baseline (Screening Visit of Study NS-065/NCNP-01-201) and the Investigator determines that the abnormality is related to investigational product, the abnormality will be discussed with the Medical Monitor. The ECG abnormality will be monitored and evaluated with additional tests (if necessary) until the underlying cause is determined or the event is brought to an acceptable resolution. Additional clinical and laboratory information will be collected and carefully documented in order to better characterize the ECG abnormality and rule out alternative causes. ECG findings determined to be a clinically significant change from baseline should be reported as an adverse event regardless of causality.

Unscheduled ECG assessments will be completed at the discretion of the Investigator.

9.13 Intravenous (IV) Access Considerations

Investigational product dosing will be administered through IV infusion. Peripheral venous access (IV catheter that empties into a peripheral vein in the arms, hands, legs or feet) is the preferred route of IP administration for this study.

Central venous access (IV catheter that empties into a large central vein) will be considered on a case-by-case basis for participants who have difficulty with peripheral venous access. An implantable central venous access (CVA) port is the preferred option of central venous access, if necessary, for this study. The Sponsor will decide whether or not to approve this option after discussions with the site PI, Study Chair and Medical Monitor have ensured mutual agreement that central venous access will still maintain a positive benefit/risk ratio for the participant in this study. Before final decision, NS Pharma will obtain documentation from the site investigator that the consulting surgeon who will place the port holds hospital privileges as a board eligible/board certified surgeon. The decision, rationale and conclusion regarding the maintained positive benefit/risk ratio will be detailed in writing and sent to the requesting site. Central venous access should not be implemented without Sponsor approval.

An alternative method of central venous access may only be considered in the case of a contraindication, in the opinion of the consulting surgeon for the placement of a totally implantable central venous access device (port).

9.14. Medical Monitor

The Medical Monitor (MM, in the SMP also referred to as MSO) in this study will assist the Sponsor and clinical sites with the review, assessment and reporting of adverse event cases, and the discussion of safety reporting issues and application of stopping rules as needed. The MM will interact with the Sponsor's pharmacovigilance team and the study sites to gain a full understanding of the reported cases to ensure the assessment and narrative of AE cases is accurate and captures the appropriate medical detail of the event for recording and reporting purposes. The MM will work closely with the Study Chair and the DSMB as appropriate.

9.15. Data and Safety Monitoring Board

CINRG has a standing DSMB that is made up of at least:

- Two medical doctors with experience in DMD;
- One medical doctor who is familiar with the interventions and the anticipated adverse events;
- A statistician; and
- A patient advocate.

It is the responsibility of the CINRG DSMB to review data quality, relevant safety data and AEs for all patients enrolled in the study and to make recommendations to the Study Chair, Medical Monitor and Sponsor regarding the ongoing conduct and monitoring of the study.

The DSMB will first meet to review and approve the study protocol. The DSMB will then meet every 6 months or as needed. Additional DSMB reviews may be requested by the Study Chair, Medical Monitor or Sponsor, as needed. DSMB meetings may be held via teleconference as necessary.

A summary safety report will be produced every 6 months in collaboration between the clinical CRO, data management group and statistician and provided to the DSMB for review. The report will include safety data and study progress data (enrollment, data completion and data quality). Upon completion of safety data reviews, the DSMB may recommend revision or modification to

the study (e.g., change in eligibility criteria, revision of informed consent). The DSMB may also assist the Sponsor to evaluate regulatory reporting requirements of an event or group of events.

10. PLANNED STATISTICAL METHODS

10.1 General Considerations

The statistical analyses described in this section will be performed as further outlined in the Statistical Analysis Plan (SAP), which will be finalized prior to database lock. The SAP will supersede the protocol if there are any differences between the two documents in the plans for data analysis and the differences will be noted in the SAP. The SAP will be included as an appendix in the clinical study report for this protocol. Statistical analyses will be performed using SAS 9.4 or higher.

Patients will be enrolled in this study after completing Study NS-065/NCNP-01-201 and will continue with the study dose (40 mg/kg/week or 80 mg/kg/week) they received in Study NS-065/NCNP-01-201. Results for this study will be summarized by study dose.

Statistical analyses will occur at the following times:

- Every 6 months. A report for the DSMB will be produced including all safety data available at the temporary data lock. A template report will be provided to the DSMB prior to the first report, and the report formats will be updated at the DSMB's requests as the data develop. The DSMB will review completeness of efficacy data, but will not review the efficacy data itself.
- At the conclusion of the study. A summary report will be generated on all baseline characteristics, safety assessments and efficacy assessments, addressing each of the primary, secondary and exploratory objectives of the study.

All statistical tests will be performed at a two-sided significance level of 0.05 with no corrections for multiple comparisons or multiple outcomes. Note however, that since this is an early phase extension study, with a focus on safety, the emphasis will not be on statistical tests (i.e., p-values).

10.2 Determination of Sample Size

Approximately 16 patients from Study NS-065/NCNP-01-201 will enter this study. Because this is an open-label extension study conducted in patients who complete Study NS-065/NCNP-01-201, the sample size is not based on any statistical considerations.

10.3 Analysis Populations

All analyses will be based on the actual treatment each patient received. Two populations will be defined for data analysis: the Safety Population and the Full Analysis Set.

Safety Population

All patients who receive at least one dose of NS-065/NCNP-01 Injection 250 mg in both the dose-finding (NS-065/NCNP-01-201) and extension study (NS-065/NCNP-01-202) will be included in the Safety Population. The Safety Population is the primary analysis population for safety assessments.

Full Analysis Set (FAS)

All patients who receive at least one dose of NS-065/NCNP-01 Injection 250 mg in both the dose-finding (NS-065/NCNP-01-201) and extension study (NS-065/NCNP-01-202) will be included in the FAS. The FAS is the primary analysis population for clinical efficacy and exploratory PD assessments.

10.4 Demographics and Baseline Characteristics

Summaries of patient demographics (age, race, ethnicity and dominant hand), baseline safety characteristics (anthropometrics, vital signs, physical examination, hematology, chemistry, BNP, urinalysis, ECG and antibodies), and baseline efficacy parameters will be done by dose level. Note that two sets of baseline values will be summarized, Screening Visit (from Dose-finding Study NS-065/NCNP-01-201) and Week 25 (from Extension Study NS-065/NCNP-01-202).

Any differences in distribution of baseline characteristics will be noted, although with such a small study, it is to be expected that some differences will emerge.

10.5 Safety Assessments

Safety analyses will be performed using the safety population and will address one of two co-primary objectives of the study. All safety assessments will be based on actual treatments received by participants.

10.5.1 Anthropometrics, Vital Signs, Laboratory Assessments, and ECG

Anthropometrics, vital signs, hematology, chemistry, BNP, urinalysis, and ECG results will be summarized by dose level over time using descriptive statistics for continuous outcomes. Actual values and change from Screening Visit (hematology, chemistry and urinalysis) or Day 1 (anthropometrics, vital signs and ECG) from Study NS-065/NCNP-01-201 and change from Week 25 (baseline of the NS-065/NCNP-01-202 study) will be presented. Further, all lab abnormalities will be listed.

10.5.2 Physical Exam and Adverse Events

Physical exam results will be summarized by frequency of presence of abnormalities in body system (beyond the DMD diagnosis) and in particular any changes in the physical exam over time.

Any new adverse events (AEs) occurring after the time of enrollment into NS-065/NCNP-01-202 (defined as the time of IP infusion at the Week 25 visit) will be considered for analysis for this study. For AEs starting in study NS-065/NCNP-01-201 which are not resolved at the time of enrollment into NS-065/NCNP-01-202, any change in outcome or relatedness will be reported in study NS-065/NCNP-01-201. For AEs starting in study NS-065/NCNP-01-201 which increase in severity or becomes serious after enrollment in study NS-065/NCNP-01-202, a new AE will be reported in NS-065/NCNP-01-202.

Treatment-emergent AEs (TEAEs) will be summarized by dose level. Coding will be done by system organ class and preferred term (using the Medical Dictionary for Regulatory Activities (MedDRA)). Level of severity will be assessed using the CTCAE grading system. Summaries will include:

A. Summaries at the patient level

How many patients had any TEAE, any SAE, highest severity of TEAE within a patient across all infusions, highest relationship level of TEAE within a patient across all infusions, highest intervention level regarding investigational product (e.g., discontinued, vs. reduced dose vs. temporarily stopped vs. no interruption in infusions), and worst outcome within a patient (e.g., AE did not resolve and has a permanent effect).

B. Summary at the event level

Summaries will be done using the MedDRA coding by events and overall, summarizing by system organ class and preferred term, by relationship to investigational product, severity, intervention, and outcome.

Listings tables will be provided for all AEs.

10.5.3 Concomitant Medications and/or Other Treatments

GCs, which are required as part of the inclusion criteria, will be summarized by type of GC (prednisone vs. deflazacort), by schedule (daily vs. any other), and by dose level. Patients are required not to change the GC dose while on study. Any changes in doses or schedule will be listed.

Other concomitant medications will be summarized by ATC class and preferred term. Each medication will be counted once within a patient using it, regardless of the number of times it was reported on the eCRFs. The summaries will note new medications or supplements vs. those already given at baseline and study entry. Any other treatment, surgeries, will be listed and described; however, those are expected to be few without a need to be summarized using tables.

10.5.4 Cytokines, Antibodies and Pharmacokinetics

Cytokines and antibodies and pharmacokinetic concentrations will be summarized by dose level over time. Pharmacokinetic concentrations may be summarized at a later date. PK samples will only be analyzed in the event that anti-NS-065/NCNP-01 antibodies are detected in order to understand the impact of immunogenicity on plasma levels of the drug.

10.6 Efficacy Endpoints

All efficacy analyses will be performed using the FAS population. In addition, additional analyses will utilize historical control group data from the CINRG DNHS.

10.6.1 Primary Efficacy Objective

The primary efficacy outcome measure (TTSTAND) will be summarized by dose level over time using descriptive statistics. Actual values and change from the Pre-Infusion Visit (baseline from Study NS-065/NCNP-01-201) and change from Week 25 (baseline of the NS-065/NCNP-01-202 study) will be presented.

The TTSTAND times to perform the test will also be converted to velocities. (Note that a test result that is to be converted to velocity that the patient could not perform due to disease progression will have velocity set to zero only at the first visit where this occurs. After that visit, missing observations due to disease progression will be left as missing.). If needed, a data transformation or nonparametric approach will be performed for these assessments. Within patient changes and velocities (possibly transformed) will be tested using a one-sided paired t-test within dose level and within both dose groups combined.

10.6.2 Secondary Efficacy Objectives

10.6.2.1 Analyses of NS-065/NCNP-01 patients only

TTRW, TTCLIMB, NSAA, 6MWT and QMT will be summarized by dose level over time using descriptive statistics. Actual values and change from the Pre-Infusion Visit (baseline from Study NS-065/NCNP-01-201) and change from Week 25 (baseline of the NS-065/NCNP-01-202 study) will be presented.

The TTRW and TTCLIMB times to perform the test will also be converted to velocities. For a test that the patient could not perform, the velocity will be set to zero. If needed, a data transformation or nonparametric approach will be performed for these assessments. Within patient changes and velocities (possibly transformed) will be tested using a one-sided paired t-test within dose group and within both dose groups combined.

10.6.2.2 Analyses comparing NS-065/NCNP-01 patients to historical controls

A matched data set from the CINRG DNHS data will be created. The purpose of the matching is to create a group data set that corresponds in characteristics to the patients in this study. The CINRG DNHS data set includes patients from age 2 years to over 30 years old, and some have been followed for close to a decade. Therefore, it is important to create a comparator group which will allow valid group comparisons. No patient to patient matched analysis is proposed. The sole purpose of the matching is to create a historical control group which is comparable in its basic characteristics to the study patient group. The final CINRG DNHS data set is expected to have between 16 and 32 patients included with time intervals of evaluations between 6 and 15 months. TTSTAND, TTRW, TTCLIMB, NSAA, 6MWT, and QMT results will be compared between the NS-065/NCNP-01 patients and the CINRG DNHS patients possibly using mixed-effects linear

models. The TTSTAND, TTRW, and TTCLIMB times to perform the test will be converted to velocities. For a test that the patient could not perform, the velocity will be set to zero.

10.7 Interim Analyses

Safety analyses are planned every 6 months as described above. Moreover, semi-annual reports to the DSMB will be descriptive and focus on safety.

In addition, interim analyses for safety are planned and interim analyses for efficacy may be conducted and further described in the Statistical Analysis Plan.

10.8 Handling of Missing Data

Every effort will be made to collect all data. However, despite best efforts, missing or incomplete data may be reported. All missing or partial data will be presented in the patient data listing, as they are recorded on the eCRF.

Patients lost to follow-up or withdrawn will be included in statistical analyses up to the point of their last evaluation. Unless otherwise specified, no imputation of values for missing data will be performed. Of note, since patients with DMD are expected to decline over time, imputing efficacy parameters by last value carried forward mostly biases towards patients appearing stronger or faster than they are, since it carries forward potentially a better value than the value at the time of the missed observation. Therefore, for this study, we will summarize how much data are missing, but do not expect to need to impute any data to accomplish the analyses as described. Details of handling missing data will be described in the Statistical Analysis Plan.

Missed assessments which occurred as a result of the COVID-19 pandemic will be entered on the COVID-19 Protocol Deviation Log by site personnel. It is anticipated that some or all assessments and laboratory tests required by the protocol schedule of events may be missed during the pandemic. Some of the missed assessments may be able to be collected at the next home or onsite visit. The missing data will be summarized in the Statistical Analysis Plan.

11 ADMINISTRATIVE CONSIDERATIONS

11.1 Investigators

The Investigator must agree to the responsibilities and obligations listed below, as specified by the appropriate FDA/Health Canada regulatory requirements or ICH/GCP guidelines:

- Agree to conduct the study in accordance with the relevant current protocol;
- Agree to personally conduct or supervise the described investigation(s);
- Agree to inform any patients, or persons used as controls, that the investigational products are being used for investigational purposes and ensure that the requirements relating to obtaining informed consent and IRB/REB review and approval are met;
- Agree to report adverse experiences that occur during the course of the investigation(s);
- Read and understand the information in the Investigator's Brochure, including the potential risks and side effects of the investigational product;
- Ensure that all associates, colleagues, and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments;
- Maintain adequate and accurate records and make those records available for inspection;
- Ensure that an IRB/REB will be responsible for the initial and continuing review and approval of the clinical investigation;
- Agree to promptly report to the IRB/REB all changes in the research activity and all unanticipated problems involving risks to patients or others;
- Agree to not make changes in the research without IRB/REB approval, except where necessary to eliminate apparent hazards to patients; and
- Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements.

Refer also to:

- FDA Regulations Related to Good Clinical Practice and Clinical Trials: http://www.fda.gov/oc/gcp/regulations.html
- Guidance and Information Sheets on GCP in FDA-Regulated Clinical Trials: http://www.fda.gov/oc/gcp/guidance.html
- Guidance for IRBs and Clinical Investigators: http://www.fda.gov/oc/ohrt/irbs/default.htm

- Guidance for Industry - E6 Good Clinical Practice: Consolidated Guidance
  http://www.fda.gov/cder/guidance/959fnl.pdf

11.2 Informed Consent, Protected Health Information (PHI) and Confidentiality

11.2.1 Informed Consent

The ICF, assent form and consent process must comply with US 21CFR Part 50 and local laws. The ICF/Assent Form will document the study-specific information provided to the patient by the investigator or designee and the patient's/legal guardian's agreement to participate in the study. The investigator, or designee (as described on Delegation of Authority log), must explain in terms understandable to the patient, the purpose and nature of the study, the study procedures, anticipated benefits, potential risks, the possible adverse effects and any discomfort participation in the study may involve. Each patient must provide a signed and dated ICF before any study related procedures are performed. In the case of a subject who is incapable of providing informed consent, the investigator or designee must obtain a signed and dated ICF from the patient's legal guardian. Minors, who are not legally capable of giving informed consent, may possess the ability to assent or dissent to participation in the study. The investigator, or designee, should explain the study and study procedures to the minor in as much detail as the minor is able to comprehend. IRB/REB-approved, age appropriate Assent Forms must be obtained from minor patients as required by local laws and governing IRB/REBs.

11.2.2 Confidentiality

Authority regulations (FDA/Health Canada) require the Sponsor or the Sponsor's authorized representative to inspect all study documents and records maintained by the Investigator, including but not limited to medical records (office, clinic, or hospital) for the patients in this study. These regulations also allow the Sponsor's records to be inspected by authorized representatives of the regulatory authorities. The names and identities of all research patients will be kept in strict confidence and will not appear on eCRFs or other records provided to or retained by the Sponsor or the Sponsor's authorized representative. Patient confidentiality will be respected during review of source documents by monitors, auditors and other Sponsor representatives. Review procedures will adhere to regulatory requirements and professional standards for confidentiality. Names and identities of patients can be protected by de-identifying (i.e., "blacking-out") patient's name and

replacing the name with the patient's study identification number. The ICF must include appropriate statements explaining these requirements.

11.2.3 Protected Health Information (PHI)

Information on maintaining patient confidentiality in accordance with US and local patient privacy regulations must be provided to each patient/legal guardian as part of the informed consent process, either as part of the ICF or as a separate signed HIPAA consent. The investigator or designee must explain to each patient that for the evaluation of study results, the patient's PHI obtained during the study may be shared with NS Pharma and its designees, regulatory agencies and IRBs/REBs. As the Study Sponsor, NS Pharma will not use the patient's PHI or disclose it to a third party without applicable patient authorization. It is the investigator's responsibility to obtain written permission to use PHI from each patient/legal guardian. If a patient or patient's legal guardian withdraws permission to use PHI, it is the investigator's responsibility to obtain the request in writing and ensure that no further data is collected on the patient. Any data collected up to the point of HIPAA consent withdrawal may be used in analysis of the study results.

11.3 Study Administrative Structure

Study Chair

Paula R. Clemens, MD
University of Pittsburgh
A506 Scaife Hall
3550 Terrace Street
Pittsburgh, Pennsylvania 15261
Phone: (412) 648-9762
Email: pclemens@pitt.edu

Medical Monitor
Helmut H. Albrecht, MD
PharmaLex Development Services, LLC
3350 SW 27th Ave, Apt. 2203
Miami, FL 33133
Phone: (973) 454-9859
Email: helmut@H2A-Associates.com

11.4 Institutional Review Board/Research Ethics Board Approval

Before initiation of the study, the Investigator must obtain approval or favorable opinion of the research protocol, informed consent form, and any material related to patient recruitment from an IRB or REB complying with the provisions specified in 21 CFR Part 56 and applicable pertinent state and federal requirements of each participating location, including International Conference on Harmonization (ICH) and Good Clinical Practice (GCP) guidelines.

Institutional Review Boards and Research Ethics Boards must be constituted according to the applicable laws. It is the responsibility of each clinical site to submit the protocol, Investigator's Brochure, patient informed consent, patient recruitment materials (if applicable), and other documentation as required by the IRB/REB for review and approval. A copy of the written approval must be provided to NS Pharma.

The documentation should clearly mention the approval/favorable opinion of the protocol, the patient informed consent form, and patient recruitment materials (if applicable), including respective version dates. The written approval and a list of the voting members, their titles or occupations, and their institutional affiliations must be obtained from the IRBs/REBs and provided to NS Pharma (or its authorized CRO) prior to the release of clinical study supplies to the clinical site and commencement of the study. If any member of the IRB/REB has direct participation in this study, written notification regarding his or her abstinence from voting must also be obtained.

Clinical sites must adhere to all requirements stipulated by their respective IRB/REB. This includes notification to the IRB/REB regarding: protocol amendments, updates to the patient informed consent, recruitment materials intended for viewing by patients, IND Safety Reports, serious and unexpected adverse events, reports and updates regarding the ongoing review of the study at intervals specified by the respective IRB/REB, and submission of final study reports and summaries to the IRB/REB.

It is the responsibility of each clinical site to submit information to the appropriate IRB/REB for annual review and annual re-approval.

The Investigator must promptly inform their IRB/REB of all SAEs or other safety information reported from the patient or NS Pharma (or the Sponsor's authorized CRO).

11.5 Ethical Conduct of the Study

The Investigator agrees, when signing the protocol, to adhere to the instructions and procedures described in the protocol and conduct the study in accordance with the CFRs (21 CFR Parts 11, 50, 54, 56, 312, 314, and 320) and local regulations, which originate from the ethical principles laid down in the current revision of the Declaration of Helsinki, GCPs, and policies and procedures as outlined by the ethical requirements for IRB/REB review and informed consent forms.

The Investigator agrees to allow monitoring and auditing of all essential clinical study documents by NS Pharma or its authorized representatives and inspection by the FDA or other appropriate regulatory authorities. Monitoring and auditing visits by NS Pharma or authorized designee will be scheduled with the appropriate staff at mutually agreeable times periodically throughout the study.

The Investigator will assure proper implementation and conduct of the study, including those study-related duties delegated to other appropriately qualified individuals. The Investigator will assure that study staff cooperates with monitoring and audits and will demonstrate due diligence in recruiting and screening study patients. The Investigator must sign and return to NS Pharma (or its authorized CRO) the "Study Acknowledgment" page and provide a copy of current curriculum vitae. For this study and all studies conducted under an IND in the United States, the Investigator must sign and return a complete Form FDA 1572 "Statement of Investigator" to NS Pharma (or the Sponsor's authorized CRO). Similarly, Investigators in Canada must sign and return the "Qualified Investigator Undertaking" form to be retained by the Sponsor.

11.6 Study Monitoring

NS Pharma (or its authorized CRO) has the obligation to follow this study closely to ensure that the study is conducted in accordance with the protocol, ICH and GCP regulatory requirements, the CFRs, FDA, and the current Declaration of Helsinki throughout its duration by means of personal visits to the Investigator's facilities and other communications.

These visits will be conducted to evaluate the progress of the study, verify the rights and well-being of the patients are protected, and verify the reported clinical study data are accurate, complete, and verifiable from source documents. This includes review of informed consent forms, results of tests performed as a requirement for participation in this study, and any other medical records (e.g.,

laboratory reports, clinic notes, investigational product disbursement log, pharmacy records, patient sign-in sheets, patient-completed questionnaires, telephone logs, ECGs) required to confirm information contained in the eCRFs.

A monitoring visit should include a review of the essential clinical study documents (regulatory documents, case report forms, medical records and source documents, investigational product disposition records, patient informed consent forms, etc.) as well as discussion on the conduct of the study with the Investigator and staff.

The monitor should conduct these visits as frequently as appropriate for the clinical study. The Investigator and staff should be available during these visits for discussion of the conduct of the study as well as to facilitate the review of the clinical study records and resolve/document any discrepancies found during the visit.

All monitoring activities will be reported and archived. In addition, monitoring visits will be documented at the clinical site by signature and date on the study-specific monitoring log.

Details of monitoring procedures will be described in the study monitoring plan and the COVID-19 study monitoring plan addendum.

11.7 On-Site Audits

Representatives of NS Pharma or its authorized clinical quality assurance group may visit a clinical site at any time during the study to conduct an audit of the study in compliance with regulatory guidelines and company policy. These audits will require access to all study records, including source documents, for inspection and comparison with the eCRFs. Patient privacy must be respected. The Investigator and clinical site personnel are responsible for being present and available for consultation during routinely scheduled site audit visits conducted by NS Pharma or its authorized representative.

The clinical study may also be inspected by the FDA (or other regulatory authorities) to verify that the study was conducted in accordance with protocol requirements, as well as the applicable regulations and guidelines.

In the event the Investigator is contacted by regulatory authorities who wish to conduct an inspection of the clinical site, the Investigator will promptly notify NS Pharma (or its authorized CRO) of all such requests and will promptly forward a copy of all such inspection reports.

Due to the global COVID-19 pandemic of 2020, on-site monitoring visits were temporarily converted to remote monitoring visits as a result of travel, site and safety restrictions. During this time, the site provided redacted source documents for monitoring verification. The monitor followed the procedures outlined in the COVID-19 study monitoring plan addendum. Once the COVID-19 travel and monitoring bans are lifted, the on-site monitoring will return to its normal schedule and procedures.

11.8 Case Report Forms and Study Records

Access to eCRFs will be provided to the clinical site. As part of the responsibilities assumed by participating in the study, the Investigator agrees to maintain adequate case histories for the patients treated as part of the research under this protocol. The Investigator agrees to maintain accurate source documentation and eCRFs as part of the case histories.

Study records are comprised of source documents, eCRFs, and all other administrative documents (e.g., IRB/REB correspondence, clinical study materials and supplies shipment manifests, monitoring logs and correspondence). A study-specific binder will be provided with instructions for the maintenance of study records.

Source documentation is defined as any hand written or computer-generated document that contains medical information or test results that have been collected for or in support of the protocol specifications (e.g., laboratory reports, clinic notes, investigational product disbursement log, pharmacy records, patient sign-in sheets, patient completed questionnaires, telephone logs, x-rays, and ECGs). All draft, preliminary, and pre/final iterations of a final report are also considered to be source documents (e.g., faxed and hard copy of laboratory reports, faxed and hard copy of initial results, and final report).

The Investigator agrees to allow direct access to all essential clinical study documents for the purpose of monitoring and/or auditing by NS Pharma or its authorized representatives and inspection by the appropriate regulatory authorities.

Data reflecting the patient's participation with the investigational product under investigation are to be reported to NS Pharma. The data are to be recorded on the eCRFs and/or other media provided or approved by NS Pharma.

A complete eCRF must be submitted for each patient who receives investigational product, regardless of duration. All supportive documentation submitted with the eCRF, such as laboratory or hospital records, should be clearly identified with the study and patient number. Any personal information, including patient name, should be removed or rendered illegible to preserve individual confidentiality. The eCRF should not be used as a source document unless otherwise specified by NS Pharma.

Neither NS Pharma nor a service provider contracted to analyze data and complete the study report is permitted to interpret a blank answer; therefore, all fields should be complete. All requested information must be entered on the eCRFs. If an item is not available or is not applicable, this fact should be indicated as (N/A) not available or (N/D) not done; do not leave a space blank.

It is essential that all dates appearing on NS Pharma patient data collection forms for laboratory tests, cultures, etc., be the dates on which the specimens were obtained, or the procedures performed. The eCRFs will be electronically signed by the Investigator and dated as verification of the accuracy of the recorded data. All data collection forms should be complete within 48 hours following the evaluation.

11.9 Amendments

Changes to the research covered by this protocol must be implemented by formal protocol amendment. All amendments to the protocol must be initiated by NS Pharma and signed and dated by the Investigator. Protocol amendments must not be implemented without prior IRB/REB approval. Documentation of amendment approval by the Investigator and IRB/REB must be provided to NS Pharma or its authorized CRO. When the change(s) involve only logistic or administrative aspects of the study, the IRB/REB only needs to be notified.

During the course of the study, in situations where a departure from the protocol is unavoidable, the Investigator will contact the NS Pharma Medical Monitor and the Study Chair. Except in emergency situations, this contact should be made before implementing any departure from the protocol. In all cases, contact with the Medical Monitor and Study Chair must be made as soon as

possible to discuss the situation and agree on an appropriate course of action. The data recorded on the eCRF and source documents will reflect any departure from the protocol and the source documents will describe the departure and the circumstances requiring it.

11.10 Access to Source Documentation

Authority regulations require the Sponsor (or the Sponsor's authorized representative) to inspect all documents and records to be maintained by the Investigator, including but not limited to, medical records (office, clinic, or hospital) for the patients in this study. These regulations also allow the Sponsor's records to be inspected by authorized representatives of regulatory authorities. The Investigator will permit study-related monitoring, audits, IRB/REB review, and regulatory inspections by providing direct access to source data/documents. Direct access includes permission to examine, analyze, verify, and reproduce any records and reports that are important to the evaluation of a clinical study.

11.11 Record Retention

In compliance with the ICH/GCP guidelines, the Investigator/Institution agrees to retain and maintain all study records that support the data collected from each patient, as well as all study documents as specified in ICH/GCP, Section 8 Essential Documents for the Conduct of a Clinical Trial. The Investigator agrees to contact NS Pharma before destroying or relocating any study documentation and is expected to take measures to prevent accidental or premature destruction of these documents.

If the Investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept responsibility. NS Pharma must be contacted in writing regarding the name and address of the new person responsible as well as the disposition of document storage. Under no circumstances shall the Investigator relocate or dispose of any study documents before having obtained written approval from NS Pharma.

Essential records (including eCRFs, source documents, clinical drug disposition records, signed patient informed consent forms, adverse event reports, and other regulatory documents) as required by the applicable regulations, must be maintained for 2 years after a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is

to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and the FDA is notified.

It is the responsibility of NS Pharma or its authorized CRO to inform the Investigator/Institution as to when these documents no longer need to be retained.

11.12 Financial Disclosure

Prior to the start of the study, Investigators will release sufficient and accurate financial information that permits NS Pharma to demonstrate that an Investigator and all study personnel listed on the FDA Form 1572 or the Health Canada Qualified Investigator Undertaking form have no personal or professional financial incentive regarding the future approval or disapproval of the investigational product such that his or her research might be biased by such incentive.

11.13 Publication and Disclosure Policy

It is understood by the Investigator that the information and data included in this protocol may be disclosed to and used by the Investigator's staff and associates as may be necessary to conduct this clinical study.

All information derived from this clinical study will be used by NS Pharma (or designee) and therefore, may be disclosed by NS Pharma (or designee) as required to other clinical investigators, to the FDA, and to other government agencies, or in connection with intellectual property filings or publications. In order to allow for the use of the information derived from this clinical study, it is understood by the Investigator that there is an obligation to provide NS Pharma with complete test results and all data from this clinical study. The Investigator agrees to maintain this information in confidence, to use the information only to conduct the study and to use the information for no other purpose without NS Pharma's prior written consent (or as otherwise may be permitted pursuant to a written agreement with NS Pharma or its designee).

The results of the study will be reported in a clinical study report (CSR) prepared by NS Pharma (or designee), which will contain eCRF data from all clinical sites that conducted the study.

Patient identifiers will not be used in publication.

NS Pharma shall have the right to publish data from the study without approval from the Investigator. Manuscript(s) and abstract(s) may only be prepared through cooperation between NS

Pharma (or designee) and the study investigator(s). If an investigator wishes to publish information from the study, a copy of the manuscript must be provided to NS Pharma for review in accordance with the provisions of such investigator's written agreement with NS Pharma (or designee) before submission for publication or presentation. If requested by NS Pharma in writing, the investigator will withhold such publication in accordance with the provisions of such agreement.

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Appendix 1 Sponsor Signatures

Study Title:	A Phase II, Open-Label, Extension Study to Assess the Safety and Efficacy of NS-065/NCNP-01 in Boys with Duchenne Muscular Dystrophy (DMD)
Study Number:	NS-065/NCNP-01-202
Final Date:	21Jul2020

This clinical study protocol was subject to critical review and has been approved by the Sponsor. The following personnel contributed to writing and/or approving this protocol:

Signed: _____ Date: _____
Tatsuya Horiguchi
Senior Director, Clinical Research
NS Pharma, Inc.

Signed: _____ Date: _____
Paula R. Clemens, MD
Study Chair
Professor of Neurology
University of Pittsburgh

Signed: _____ Date: _____
Helmut H. Albrecht, MD, MS, FFPM
Medical Monitor
PharmaLex Development Services, LLC

Signed: _____ Date: _____
Mark J. Jaros, PhD
Statistician
Summit Analytical, LLC

Appendix 2 Investigator's Signature

Study Title:	A Phase II, Open-Label, Extension Study to Assess the Safety and Efficacy of NS-065/NCNP-01 in Boys with Duchenne Muscular Dystrophy (DMD)
Study Number:	NS-065/NCNP-01-202
Final Date:	21Jul2020

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

Signed:_____ Date:_____

<enter name and credentials>
<enter title>
<enter affiliation>
<enter address>
<enter phone number>

Appendix 3 Summary of Changes

Amendment 1 Changes to the Protocol:

Updates to the protocol include the following changes:

1. Change study site locations to North America
2. Include Research Ethics Boards (REB) in addition to IRBs
3. Include references to Health Canada where applicable
4. Allow additional patients to enroll beyond 8 in each cohort

Change	Rationale	Affected Area
Added Amendment 1 and date.	Identify amendment 1.	Title page Header throughout.
Update study site locations to North America.	Include participation of Canadian sites.	Title Page Synopsis Clinical Sites.
Addition of Research Ethic Boards.	Update terminology to apply to Canadian sites.	Document throughout.
Addition of Health Canada and applicable regulations.	Include participation of Canadian sites.	Document throughout.
Addition of brain natriuretic protein (BNP) lab collection.	Allows collection of cardiac function data in this long-term exposure to the investigational drug.	Section 7.11.1. Sample Collection, Storage, and Shipping. Table 2 Clinical Laboratory Tests. Section 8.1.1. Day of First Infusion (Week 25). Section 8.2.1 Week 49 or Early Termination. Table 3 Blood Sample Volume and Number.

Amendment 2 Changes to the Protocol:

Updates to the protocol include the following changes:

1. Added information regarding EMFLAZA™ approval by the FDA
2. Increase weekly IP infusion timeline from 48 weeks to 96 weeks
3. Add additional site locations for infusion visits
4. Combine week 48 blood draw assessments to week 49
5. Provided clarification of the minimum time that must elapse between infusions
6. Clarified details of height measurements
7. Clarified analysis of adverse events that occur during study NS-065/NCNP-01-201 and are not resolved prior to enrollment into this study
8. Clarified language regarding collection times for PK samples relative to infusion start time
9. Clarified language regarding collection times for vital signs relative to infusion start time

Change	Rationale	Affected Area
Added Amendment 2 and date.	Identify amendment 2	Title page Header throughout.
Added information regarding EMFLAZA™ approval.	Provide more complete information on DMD management.	Section 1.2.1. Glucocorticoid Treatment.
Increase infusion timeline from 48 weeks to 96 weeks.	Streamline extension protocols by combining this 24-week extension protocol with a proposed 48-week long-term extension protocol.	Document throughout.
Update number of participating sites.	Include participation of regional sites for infusion visits to reduce participant travel burden.	Title Page Synopsis, Clinical Sites Section 8.1. Treatment Phase
Move week 48 blood draws to week 49.	Reduce participant travel burden. Reduce the number of venipunctures.	Document throughout.
Change Instructions from a minimum of 2 days to a minimum of 3 days (72 hours) between infusions	Provide clarification of intention.	Section 5.3. Instructions for Administration of Investigational Product
Added details of height measurements.	Clarification of procedure.	Section 7.6. Weight and Height.
Added language for analysis of AEs that occur in study NS-065/NCNP-01-201 and are not resolved upon enrollment into this study.	Provide clarification for AE analysis.	Section 10.5.2. Physical Exam and Adverse Events

Updated PK collection time language.	Provide clarification of procedure times.	7.14.1 Collection and Assessment of Pharmacokinetic Samples, Table 1 Schedule of Study Assessments
Updated Vital signs collection time language.	Provide clarification of procedure times.	7.7 Vital Signs, Table 1 Schedule of Study Assessments

Amendment 3 Changes to the Protocol:

Updates to the protocol include the following changes:

1. Clarified procedure for determining visit windows
2. Added language to clarify that blood sample collection should not occur at the same venous site as IP infusion
3. Corrected blood total volume by removing Early Termination samples from total
4. Corrected order of assessments at each study visit
5. Clarified differences between procedures at Week 97 visit and Early Termination Visit
6. Included option for central venous access for participants who experience difficulty with peripheral intravenous access

Change	Rationale	Affected Area
Added Amendment 3 and date.	Identify amendment 3	Title page Header throughout.
Included sentence regarding weekly visit windows.	To clarify that all visits should be calculated from 1st infusion of NS-065/NCNP-01-201.	Section 5.5. Treatment Compliance
Added language regarding blood sampling during IP infusion.	To clarify any blood sampling occurring during IP infusion must be collected from a site away from IP infusion.	Section 7.11. Clinical Laboratory Tests
Corrected blood total volume collected	Early Termination volumes should not be included in total	Section 7.15. Total Blood Volume of Clinical Laboratory and Pharmacokinetic Samples Table 3. Blood Sample Number and Volume
Corrected Order of Assessments at each study visit.	To accurately reflect the order in which study procedures should be performed.	Section 8.1. Treatment Phase Section 8.2. Post-Treatment Phase
Created separate sections for Week 97 and Early Termination Visit	To clarify differences between procedures at these visits. Early Termination visit includes PK, Cytokines and Antibodies. These procedures are not done at Week 97	Section 8.2.1. Week 97 Section 8.2.2. Early Termination Visit
Added option for use of central line for weekly study infusions	The placement of peripheral intravenous lines has been very difficult for some participants. These	Section 3.2.2. Investigational Product Dosing Section 9.12. Intravenous (IV) Access Considerations

	difficulties add additional stress to the participant and families. Site PIs have expressed concern about continual weekly access and the potential for participants to miss study infusions or discontinue participation.	

Amendment 4 Changes to the Protocol:

Updates to the protocol include the following changes:

1. Revised to specify "implantable ports" may be used for this trial. An alternative method of central venous access may only be considered in the case of a contraindication.
2. NS Pharma must obtain surgeon credentials prior to port placement.
3. Included reporting of serious adverse events suspected to be related to the port to the FDA and Health Canada

Change	Rationale	Affected Area
Added Amendment 4 and updated version date.	Identify Amendment 4	Title page Appendix 1 – Sponsor Signatures Appendix 2 – Investigator Signatures Header throughout document
Added CVA to list of abbreviations	Identify the abbreviation and provide definition	List of Abbreviations
Specified "implantable central venous access ports"	An implantable port is the preferred option of the sponsor for central venous access given the reported lower infection rates and lower replacement rates.	Section 3.2.2. Investigational Product Dosing Section 9.12. Intravenous (IV) Access Consideration
Sites that request use of implantable port must provide documentation of consulting surgeon's hospital privileges as a board eligible/board certified surgeon.	To be consistent with the sponsor's rationale for continued positive benefit/risk ratio with implantable ports.	Section 3.2.2. Investigational Product Dosing Section 9.12. Intravenous (IV) Access Consideration
Indicated that alternative methods of CVA will only be considered in cases of contraindication.	Although, an implantable port is the preferred option, the sponsor may allow alternative methods based on the best judgement of site PI and consulting surgeon.	Section 3.2.2. Investigational Product Dosing Section 9.12. Intravenous (IV) Access Consideration
Indicated the sponsor will report any serious adverse events suspected to be related to the CVA port to the FDA and HC	Risks associated with implantable CVA ports can become a serious complication. Documentation and notification will ensure continued positive benefit/risk ratio of the study.	Section 9.7 Reporting of Serious Adverse Events to Regulatory Authorities

Amendment 5 Changes to the Protocol:

Updates to the protocol include the following changes:

1. Revised to include new concentration of investigational product (IP).
2. Revised the study visit window to +/- 3 days.
3. Revised to not allow IP infusions at regional centers when participant weight needs to be collected.

Change	Rationale	Affected Area
Added Amendment 5 and updated version date.	Identify Amendment 5	Title page Appendix 1 – Sponsor Signatures Appendix 2 – Investigator Signatures Header throughout document
Added new concentration of IP (50 mg/mL)	New strength of IP is 250 mg vial in 5mL saline. Both strengths need to be retained since the original strength and new strength will both be used during the course of this study.	Study Synopsis Section 5.1 Description of NS-065/NCNP-01
Updated study visit windows to +/- 3 days	To accommodate participant and site schedules as this study requires weekly site visits over 72 weeks. 72-hours between infusions shall be maintained for safety reasons, as stated in the protocol.	Section 5.5 Treatment Compliance Table 1 Schedule of Study Assessments
Removed “weight” as a study procedure allowed to be done at regional infusion centers	To maintain consistency in proper weight collection and to have more consistent PI oversight by requiring participants to return to central study site more often.	Section 8.1 Treatment Phase

Amendment 5.1 Changes to the Protocol:

Updates to the protocol include the following changes:

- Corrected "Amendment 5.0 Changes to the Protocol"

Change	Rationale	Affected Area
Added Amendment 5.1 and updated version date.	Identify Amendment 5.1	Title page Appendix 1 – Sponsor Signatures Appendix 2 – Investigator Signatures Header throughout document
Removed item #4 from list of Amendment 5.0 Changes	Language indicating BNP lab sample could be collected with lab samples collected for the NS-065/NCNP-01-201 Week 25 study visit was not removed from the protocol.	Appendix 3, Summary of Changes; "Amendment 5.0 Changes to the Protocol"

Amendment 6 Changes to the Protocol:

Updates to the protocol include the following changes:

1. Revised to replace possibility of regional infusions with home infusions.

Change	Rationale	Affected Area
Added Amendment 6 and updated version date.	Identify Amendment 6	Title page Appendix 1 – Sponsor Signatures Appendix 2 – Investigator Signatures Header throughout document
Changed number of study sites from 25 to 6	The accurate number of study sites participating in this trial is 6. No additional sites will participate.	Title page Study Synopsis
Replaced possibility of regional infusions with home infusions	To offer a more convenient option for IP infusions	Section 8.1 Treatment Phase
Removed paragraph detailing the W25 visit window as +/- 3 days	Paragraph was now unnecessary as all study visit windows were updated to +/- 3 days.	Study Synopsis Section 8.1.1. Day of First Infusion (Week 25)
Updated wording about length of infusion to describe "approximately 1-hour".	To account for variability in IV pump rate.	Section 7.14.1 Collection and Assessment of Pharmacokinetic Samples
Updated to include central pharmacy of home infusion company as a recipient of IP and IP accountability	Home infusion company will store drug at their central pharmacy and ship to participants' homes as needed for weekly infusions	Section 5.0 Investigational Product
Identified which visits may be home infusion visits on Schedule of Procedures	To identify which visits may be done in a home setting.	Table 1
Corrected Time to Stand point scale	Time to Stand point scale is a 6-point scale	Section 7.13.1 Time to Stand (TTS) – Primary Efficacy Endpoint

Amendment 7 Changes to the Protocol:

Updates to the protocol include the following changes:

1. Extended study to 168 weeks or until IP is commercially available

Change	Rationale	Affected Area
Added Amendment 7 and updated version date.	Identify Amendment 7	Title page Appendix 1 – Sponsor Signatures Appendix 2 – Investigator Signatures Header throughout document
Increase infusion timeline from 96 weeks to up to 168 weeks or until study drug is commercially available.	To make study IP available to study participants until commercially available.	Document throughout and schedule of assessment
Interim analyses may be conducted	Interim review of safety and efficacy data	Synopsis Section 10.7: Interim Analyses
Updated wording regarding post-dose vitals	Clarification needed for sites and for re-collection of visits in the event of any changes from pre-dose vitals	Section 7.7: Vital Signs
Clinical laboratory tests	Format updates	Table 2
Updated wording for PK samples collection	Clarification needed for sites	Section 7.14.: Pharmacokinetic Assessments
Blood volume increase	Increase volume due to extension until up to 168 weeks	Section 7.15: Total Blood Volume and Table 3
Updated description of study visits	Study timeline increased to up to 168 weeks and combined visits that are alike	Section 8.1.: Treatment Phase
Added criteria for home infusion	Increase safety	Section 8.1.: Treatment Phase
Removed language about further long-term study	There will be no further long-term study as this study will make IP available until commercially available.	Section 8.2.3.: Follow-up Phone Call
Added reason for participant withdrawal, “The study drug becomes commercially available”	The sponsor wants to make IP available to participants until commercially available	Section 8.2.4.: Early Termination or Withdrawal from Study

Amendment 8 Changes to the Protocol:

Updates to the protocol include the following changes:

1. Extended study to 193 weeks or until IP is commercially available
2. Updated SAE reporting to a new central pharmacovigilance team at IQVIA

Change	Rationale	Affected Area
Added Amendment 8 and updated version date.	Identify Amendment 8	Title page Appendix 1 – Sponsor Signatures Appendix 2 – Investigator Signatures Header throughout document
Extended study from 168 weeks to up to 192 weeks or until study drug is commercially available.	To make study IP available to study participants until commercially available.	Synopsis, document throughout and Table 1
Removal of statement that certain P1/2 and P2 studies are still ongoing	P1/2 in Japan and P2 (Study 201) are both completed	Section 1.3.5.2 Phase II Trials
Blood volume increase	Increase volume due to extension until up to 193 weeks	Section 7.15: Total Blood Volume and Table 3
Updated SAE Event Reporting	Investigators will be notified by the CRO of all SAEs	Section 9.5
Updated SAE reporting vendor	SAE reporting changed from PharmaLex to IQVIA	Section 9.7

Amendment 9 Changes to the Protocol:

Updates to the protocol include the following changes:

1. Added pre-clinical toxicology findings
2. Added Urine Cytology assessments
3. Added Renal and Urinary Bladder Ultrasound assessments
4. Revised language throughout to correspond with language in NS-065/NCNP-01-301 protocol

Change	Rationale	Affected Area
Added Amendment 9 and updated version date	Identify Amendment 9	Title page Appendix 1 – Sponsor Signatures Appendix 2 – Investigator Signatures Header throughout document
Added additional pre-clinical toxicology findings	New pre-clinical toxicology findings	Section 1.3.4
Added language regarding dose interruptions	Language aligns with NS-065/NCNP-01-301 protocol	Section 3.2.4
Updated time IP preparation instructions	Change based on updated stability data	Section 5.3
Added Renal and Urinary Bladder Ultrasound	Safety assessment added based on new pre-clinical toxicology findings	Table 1 Section 7.9 Section 8.1.6 Section 8.1.7 Section 8.2.1 Section 8.2.2
Updated 12-lead ECG	Updated who may interpret ECG read out	Section 7.11
Updated Urinalysis from *random* collection to *pre-* and *post-dose* collections at specified visits	Collection of pre- and post-dose urinalysis specimens align with NS-065/NCNP-01-301 protocol	Table 1 Table 2 Section 8.1.6 Section 8.1.7
Added urine cytology	Safety assessment added based on new pre-clinical toxicology findings	Table 1 Section 7.12.1 Table 2 Section 8.1.6 Section 8.1.7 Section 8.2.1 Section 8.2.2
Added Adverse Events of Special Interest	Added adverse events which are considered special interest, which aligns with	Section 9.5.3

	NS-065/NCNP-01-301 protocol	
Added Urine Analysis Monitoring section	Added Monitoring of Urine Analysis verbiage to align with NS-065/NCNP-01-301 protocol	Section 9.11
Removed PK assessment	PK blood collection unnecessary at week 192	Section 8.1.7

Amendment 10 Changes to the Protocol:

Updates to the protocol include the following changes:

1. Extended study to 217 weeks or until patients transition to a long-term follow-up program of NS-065/NCNP-01.
2. Increase patients in the low dose cohort group from 40mg/kg/week to 80mg/kg/week.
3. Added details regarding the COVID-19 pandemic and how it affected the conduct of the study.

Change	Rationale	Affected Area
Added Amendment 10 and updated version date	Identify Amendment 10	Title page Appendix 1 – Sponsor Signatures Appendix 2 – Investigator Signatures Header throughout document
Extended study from 192 weeks to up to 217 weeks	To make study IP available to study participants until a long-term follow-up program is available.	Synopsis, document throughout, Tables 1 and 3
Increase 40mg/kg/week low cohort dose patients to 80mg/kg/week	All patients to be transitioned to 80mg/kg/week per advisement of FDA and to align with Japan package insert label approval.	Synopsis Section 3.1 Section 3.2.2.1 Section 5.2
Blood volume increase	Increase volume due to extension until up to 217 weeks	Section 7.16: Total Blood Volume and Table 3
COVID-19 Pandemic impact to study	Added details surrounding actions taken in response to the global COVID-19 pandemic.	Section 3.4 Section 10.8 Section 11.6 Section 11.7
Added viltolarsen to description of study drug NS-065/NCNP-01	Updated investigational study drug product with generic drug name	Synopsis
Deleted Early Termination section	Visit Week 217 and Early Termination (ET) are identical thus eliminated redundancy.	Section 8.2.1 Section 8.2.2
Updated Study Signatories	Changed signatory for NS Pharma due to personnel change	Appendix 1