Clinical Study Protocol: NS-065/NCNP-01-201

Study Title:	A Phase II, Dose Finding Study to Assess the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of NS-065/NCNP-01 in Boys with Duchenne Muscular Dystrophy (DMD)
Protocol Number:	NS-065/NCNP-01-201
Study Phase:	Phase II
Product Name:	NS-065/NCNP-01 Injection
IND Number:	127,474
Investigators:	Up to 12 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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Amendment 6 Date:	28Nov2017

STUDY SYNOPSIS

Study Title	A Phase II, Dose Finding Study to Assess the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of NS-065/NCNP-01 in Boys with Duchenne Muscular Dystrophy (DMD)
Protocol Number	NS-065/NCNP-01-201
Name of Sponsor	NS Pharma, Inc.
Investigative Product	NS-065/NCNP-01 Injection
Study Phase	Phase II
Indication	Treatment of Duchenne muscular dystrophy (DMD)
Objectives	Primary • 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 in ambulant boys with DMD. • To evaluate the effects of low and high IV doses of NS-065/NCNP-01 Injection on induction of dystrophin protein in muscle after 20-24 weeks of treatment measured by Western blot. • To evaluate the pharmacokinetics of low and high IV doses of NS-065/NCNP-01 Injection. Secondary • To evaluate the effects of low and high IV doses of NS-065/NCNP-01 Injection on induction of dystrophin mRNA and protein in muscle after 20-24 weeks of treatment as measured by RT-PCR for mRNA analysis and immunofluorescence staining and mass spectrometry methods for protein analysis. • To investigate the effect of low and high IV doses of NS-065/NCNP-01 Injection after 20-24 weeks of treatment on muscle strength, mobility, and functional exercise capacity, as measured by Time to Stand (TTSTAND), 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) vs. a matched natural history control group. Exploratory • 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, multiple center, 2-period, randomized, placebo-controlled, dose-finding study of NS-065/NCNP-01 Injection 250 mg administered by infusion once weekly for 20-24 weeks to ambulant boys ages 4-<10 years with DMD. Two dose level cohorts will be enrolled, low

	dose (40 mg/kg) and high dose (80 mg/kg). Period 1 of this study will be conducted in a double-blind fashion. Randomized patients will receive weekly IV infusions of NS-065/NCNP-01 or placebo for the first 4 weeks of their participation (Period 1) and NS-065/NCNP-01 by IV infusion for weeks 5-24 (20 weeks of active treatment – Period 2). Analysis of safety data from Period 1 of the low dose cohort will be completed prior to enrolling patients in the high dose cohort. Patients completing the 24-week study will be eligible for an open-label extension study (NS-065/NCNP-01-202) under a separate protocol. The study is comprised of a screening period with pre-treatment muscle biopsy and baseline measures, a 4-week randomized period (Period 1), a 20-week open label period (Period 2) and up to 30 days of follow-up for the patients who do not enter the open-label extension study.
Study Population	The study will enroll approximately 16 ambulant boys ages 4-<10 with DMD. **Inclusion Criteria:** 1. Patient's parent or legal guardian has provided written informed consent/HIPAA authorization prior to any study-related procedures and patient has provided assent appropriate for his age and developmental status; 2. Patient has a confirmed diagnosis of DMD defined as: a. Patient is male with clinical signs compatible with DMD; and b. Patient has a confirmed DMD mutation(s) in the dystrophin gene that is amenable to skipping of exon 53 to restore the dystrophin mRNA reading frame including determination of unambiguous defined exon boundaries (using techniques such as Multiplex Ligation-dependent Probe Amplification (MLPA), Comparative Genomic Hybridization (CGH) array or other techniques with similar capability) 3. Patient is ≥ 4 years at time of consent and <10 years of age at time of first infusion in the study; 4. Patient is able to walk independently without assistive devices; 5. Patient is able to complete the time to stand (TTSTAND), time to run walk 10 meters (TTRW) and time to climb 4 stairs (TTCLIMB) assessments as determined by the Clinical Evaluator (CE) at Screening; 6. Clinical safety laboratory test results (refer to Table 2) are within the normal range at the Screening Visit, or if abnormal, are not clinically significant, in the opinion of the Investigator; 7. Patient and parent/guardian are willing and able to comply with scheduled visits, investigational product administration plan, and study procedures;

	8. Patient must be on a stable dose of glucocorticoid (GC) for at least 3 months prior to study entry, and is expected to remain on stable dose of GC treatment for the duration of the study. **Exclusion Criteria:** 1. Patient has had an acute illness within 4 weeks prior to the first dose of study medication; 2. Patient has evidence of symptomatic cardiomyopathy. [Note: Asymptomatic cardiac abnormality on investigation would not be exclusionary]; 3. Patient has an allergy or hypersensitivity to the study medication or to any of its constituents; 4. Patient has severe behavioral or cognitive problems that preclude participation in the study, in the opinion of the Investigator; 5. Patient has previous or ongoing medical condition, medical history, physical findings or laboratory abnormalities that could affect safety, make it unlikely that treatment and follow-up will be correctly completed or impair the assessment of study results, in the opinion of the Investigator; 6. Patient is taking any other investigational drug currently or within 3 months prior to the start of study treatment; 7. Patient has had surgery within the 3 months prior to the first anticipated administration of study medication or surgery is planned for anytime during the duration of the study; 8. Patient has previously participated in this study or any other study during which NS-065/NCNP-01 was administered; 9. Patient has positive test results for hepatitis B antigen, hepatitis C antibody or HIV antibody at screening. **Note**: Any parameter/test may be repeated at the Investigator's discretion during Screening and/or Day -1 to determine sustainability and reproducibility. In addition, patients may be rescreened if ineligible due to a transient condition which would prevent the patient from participating, such as an upper respiratory tract infection.
Clinical Sites	Up to 12 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 glass vial containing 25 mg/mL of drug product in saline. Placebo will be supplied as a 10 mL matching glass vial of saline without the drug product. Patients will receive IV infusions of NS-065/NCNP-01 Injectable solution or matching placebo administered once weekly over a 4-week period, after which all patients in each dosing group will receive IV infusions of NS-065/NCNP-01 Injectable solution administered once weekly for a 20-week period. The first group of patients will be dosed at 40 mg/kg/week

	(low dose). After appropriate safety assessment, a second group of patients will be dosed at 80 mg/kg/week (high dose).
Safety Assessments	1. Overall incidence of adverse events (AEs) and serious adverse events (SAEs) 2. Changes from baseline in laboratory parameters (blood, urine) . 3. Changes from baseline in electrocardiograms (ECGs), anthropometrics, vital signs and diagnostic parameters (physical exam). 4. Anti NS-065/NCNP-01 antibodies at Week 24 compared to Day 1 5. Anti-dystrophin antibodies at Week 24 compared to Day 1 6. Cytokine levels at Week 24 compared to Day 1
Efficacy Assessments	Main: 1. Bicep muscle dystrophin protein levels at 24 Weeks compared to Pre-Infusion Visit by Western blot Secondary: 1. Bicep muscle dystrophin protein levels at 24 Weeks compared to Pre-Infusion Visit by mass spectrometry and immunofluorescence staining methods 2. Bicep muscle dystrophin mRNA levels at 24 Weeks compared to Pre-Infusion Visit 3. Time to stand (TTSTAND) at 13 and 24 Weeks compared to Pre-Infusion Visit 4. Time to run/walk 10 meters (TTRW) at 13 and 24 Weeks compared to Pre-Infusion Visit 5. Time to climb 4 stairs (TTCLIMB) at 13 and 24 Weeks compared to Pre-Infusion Visit 6. North Star ambulatory assessment (NSAA) at 13 and 24 Weeks compared to Pre-Infusion Visit 7. Six Minute Walk Test (6MWT) at 13 and 24 Weeks compared to Pre-Infusion Visit 8. Quantitative muscle testing (QMT) at 13 and 24 Weeks compared to Pre-Infusion Visit
Pharmacokinetic Assessments	NS-065/NCNP-01 level in plasma at Day 1, Week 5, Week 13 and Week 24.
Exploratory Assessments	Blood will be collected for serum PD biomarkers to explore the effect of NS-065/NCNP-01 on muscle cellular pathology.
Statistical Methods	The safety population will consist of all randomized patients who received at least 1 dose of investigational product. Patients will be analyzed as treated. This will be the primary analysis population for the evaluation of exposure and safety.

	The modified Intent-to-Treat (mITT) population will consist of all randomized patients who received at least 1 dose of investigational product and have a baseline assessment and at least 1 post baseline efficacy assessment. Patients will be analyzed as randomized. This will be the analysis population for the evaluation of efficacy. The PK concentration population will consist of all randomized patients who received at least 1 dose of investigational product and have at least 1 plasma concentration value for investigational product. All statistical tests will be performed at a significance level of 0.05 with no corrections for multiple comparisons or multiple outcomes. For addressing the primary safety objective of the study, treatment emergent adverse events (TEAEs) will be summarized for Period 1 by comparing low dose (N=6) to high dose (N=6) to placebo (N=4) and for Period 2 between the low dose cohort (N=8) and the high dose cohort (N=8). TEAEs will be summarized both at the patient level for number of TEAEs, highest severity, relationship, action and outcome and at the TEAE level (summarizing events) by organ system and preferred term TEAE as well as severity, relationship, action and outcome. The Medical Dictionary for Regulatory Activities (MedDRA) version 16.1 will be used and the Common Terminology Criteria for Adverse Events (CTCAE) grading. In addition, anthropometrics, vital signs, hematology, chemistry, urinalysis, and ECG results will be summarized descriptively. Data in Period 1 will be summarized by low dose (N=6) vs. high dose (N=6) vs. placebo (N=4). For all subsequent data collection points (Period 2), the safety assessments will be summarized by low dose enrollment cohort (N=8) vs. high dose enrollment cohort (N=8). Cytokines and antibodies will be compared for any changes over time as well as any abnormalities. For the main efficacy outcome of change in dystrophin production, within patient changes in % dystrophin production (possibly transformed) will be tested using a paired t-test within dose group and with both dose groups combined. Also, a two sample t-test to compare change across the two dose levels will be performed. The secondary efficacy outcomes of TTSTAND, TTCLIMB, TTRW, 6MWT, NSAA, and QMT assessments will be summarized descriptively at each visit using actual values and change from baseline values. The TTSTAND, TTCLIMB, and TTRW times to perform the test will also be converted to velocities.. Within patient changes (possibly transformed) will be tested using a paired difference t-test within each dose group and also with both dose groups combined. A two sample t-test to compare change across the two dose levels will also be presented.

	A matched data set from the Cooperative International Neuromuscular Research Group (CINRG) Duchenne Natural History Study (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 secondary efficacy outcome measures (TTSTAND, TTCLIMB, TTRW, 6MWT, NSAA and QMT results) will be compared between the NS-065/NCNP-01 patients and the CINRG DNHS patients using mixed-effects linear models. Pharmacokinetic parameters will be estimated with non-compartmental methods for samples obtained on Day 1 (1st dose) and Week 24. Pharmacokinetic parameters will be derived using model-independent methods and will be based on plasma concentrations of NS-065/NCNP-01 from those subjects who have received a dose of investigational product and have evaluable plasma concentration-time profiles.

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2'O-Me	2'O-methyl phosphorothioate
6MWT	6 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
Ca	Calcium
CGH	Comparative genomic hybridization
Cl	Chloride
CE	Clinical evaluator
CINRG	Cooperative International Neuromuscular Research Group
C_{max}	Maximum Drug Concentration
Cr	Creatinine
CRO	Clinical Research Organization
CS	Clinically significant
CK	Creatine kinase
Cm	Centimeter
CTCAE	Common Terminology Criteria for Adverse Events v4.03
CVC	Central Venous Catheter
CYP	Cytochrome P450
DNA	Deoxyribonucleic acid
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
HB	Hepatitis B
HCV	Hepatitis C Virus
HEENT	Head, ears, eyes, nose, and throat
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
ICF	Informed consent form
ICH	International Council of Harmonization
IND	Investigational New Drug
IPIM	Investigational Product Information Manual
IRB	Institutional Review Board
IV	Intravenous
K	Potassium
kg	Kilogram

LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
MCP-1	Monocyte chemoattractant protein-1
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified intention to treat
MLPA	Multiplex Ligation-dependent Probe Amplification
mRNA	Messenger ribonucleic acid
MS	Mass spectrometry
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 250 mg	NS-065/NCNP-01 investigational drug product (250 mg vial strength)
PHI	Protected Health Information
PI	Principal Investigator
PD	Pharmacodynamic
PK	Pharmacokinetic
PMO	Phosphorodiamidate morpholino oligomer
PT-INR	Prothrombin Time – International Normalized Ratio
QMT	Quantitative Muscle Testing
QWBA	Quantitative whole-body autoradiography
RT-PCR	Reverse transcriptase polymerase chain reaction
RBC	Red blood cell count
Ret	Reticulocyte
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 NS-065/NCNP-01-202 extension study.

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). Deflazacort was approved by the Food and Drug Administration (FDA) for the treatment of DMD under priority review on February 9, 2017.

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).

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 showing restored dystrophin as its primary outcome measure. (20) Eteplirsen (Exondys 51®) was approved by the Food and Drug Administration (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

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 in ambulant boys with DMD.
2. To evaluate the effects of low and high IV doses of NS-065/NCNP-01 Injection on induction of dystrophin protein in muscle after 20-24 weeks of treatment measured by Western blot.
3. To evaluate the pharmacokinetics of low and high IV doses of NS-065/NCNP-01 Injection.

2.2. Secondary Objectives

1. To evaluate the effects of low and high IV doses of NS-065/NCNP-01 Injection on induction of dystrophin mRNA and protein in muscle after 20-24 weeks of treatment as measured by RT-PCR for mRNA analysis and immunofluorescence staining and mass spectrometry methods for protein analysis.
2. To investigate the effect of low and high IV doses of NS-065/NCNP-01 Injection after 20-24 weeks of treatment on muscle strength, mobility, and functional exercise capacity, as measured by Time to Stand (TTSTAND), 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) vs. a matched natural history control group.

2.3. Exploratory Objectives

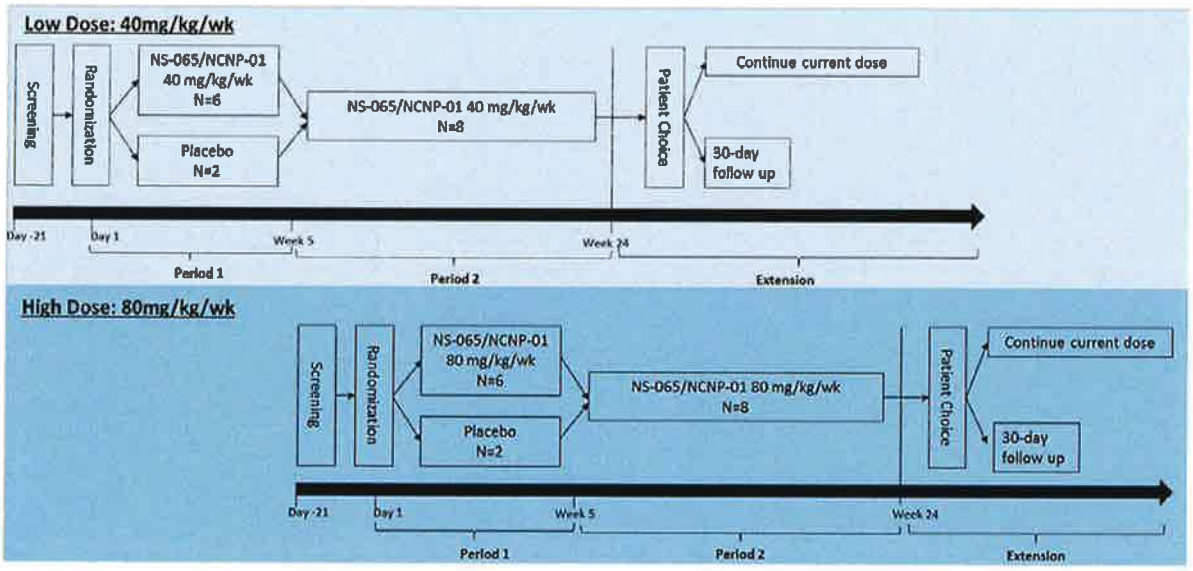
1. To investigate the effects of low and high IV doses of NS-065/NCNP-01 Injection on serum pharmacodynamic (PD) biomarkers.

3. INVESTIGATIONAL PLAN

3.1. Overall Study Design and Plan

This is a Phase II, multiple center, 2-period, randomized, placebo-controlled, dose-finding study of NS-065/NCNP-01 Injection 250 mg administered by infusion once weekly for 20 or 24 weeks to ambulant boys ages 4-<10 years with DMD.

Figure 4. Study Design



Patients completing the 24-week treatment period will be eligible for an open-label extension study under a separate protocol (NS-065/NCNP-01-202).

The study is comprised of a screening period with pre-treatment muscle biopsy and baseline measures, a 4-week randomized period (Period 1), a 20-week open label period (Period 2) and up to 30 days of follow-up for the patients who do not enter the open-label extension study.

3.2. Design Implementation

3.2.1. Randomization

The randomization schedule will be based on permuted blocks, generated by an unblinded statistician and maintained by Xerimis. All other parties will be blinded to the randomization.

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 study Investigational Product Information Manual (IPIM). Doses will be administered by an intravenous (IV) infusion over a 1-hour period. 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 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 (40mg/kg/wk)

Period 1: Eight (8) patients will be randomized in a double-blind fashion at a ratio of 3:1; where 6 patients will receive NS-065/NCNP-01 40 mg/kg/wk (low dose) and 2 patients will receive matching placebo for 4 weeks. Additional patients may be enrolled with the approval of the Study Chair and Medical Monitor. The DSMB will be informed if additional patients beyond 8 are enrolled into a cohort. Additional patients would be randomized according to the permuted block schedule. A minimum of 1 week shall elapse between the initial dosing of the first four patients to allow adequate time for safety issues to emerge. After the fourth patient has received the initial dose, subsequent patients may be dosed as enrolled. Once Period 1 safety results are

obtained for the Low Dose Cohort, the Study Chair, Medical Monitor, Data and Safety Monitoring Board (DSMB), and the sponsor will review the safety results as described in Section 9.13. If the 40 mg/kg/wk dose (low dose) is deemed safe, dosing for the next treatment group will be escalated to 80 mg/kg/wk dose (high dose). If escalation to 80 mg/kg for the next treatment group is not approved, consideration will be given to reducing the dose, as determined by the Study Chair, Medical Monitor and DSMB.

Period 2: Starting at Week 5, all patients enrolled in the Low Dose Cohort will receive open-label NS-065/NCNP-01 at 40 mg/kg/wk for 20 weeks. Thus, in the Low Dose Cohort, 6 patients will receive a total of 24 weeks of 40 mg/kg/wk NS-065/NCNP-01 and 2 patients will receive a total of 20 weeks of 40 mg/kg/wk NS-065/NCNP-01 although additional patients could be randomized in either treatment group.

3.2.2.2. High Dose Cohort (80 mg/kg/wk)

Dosing at the 80 mg/kg/wk dose (high dose) will not begin until all patients in the Low Dose Cohort have completed Period 1 and a review of the safety data has been performed by the Study Chair, Medical Monitor, DSMB, and sponsor as described in Sections 3.2.2.1 and 9.13.

Period 1: Eight (8) patients will be randomized in a double-blind fashion at a ratio of 3:1; where 6 patients will receive NS-065/NCNP-01 80 mg/kg/wk (high dose) and 2 participants will receive matching placebo for 4 weeks. Additional patients may be enrolled with the approval of the Study Chair and Medical Monitor. The DSMB will be informed if additional patients beyond 8 are enrolled into a cohort. Additional patients would be randomized according to the permuted block schedule. A minimum of 1 week shall elapse between the initial dosing of the first four patients to allow adequate time for safety issues to emerge. After the fourth patient has received the initial dose, subsequent patients may be dosed as enrolled. Once Period 1 safety results are obtained for the High Dose Cohort, the Study Chair, Medical Monitor, DSMB, and the sponsor will review the safety results as described in Section 9.13. If cumulative safety results do not warrant continuation at this dose level, the dose may be modified as described in Section 3.2.3.

Period 2: Starting at Week 5, all patients enrolled in the High Dose Cohort will receive open-label NS-065/NCNP-01 at 80 mg/kg/wk for 20 weeks. Thus, in the High Dose Cohort, 6 patients will receive a total of 24 weeks of 80 mg/kg/wk NS-065/NCNP-01 and 2 participants will

receive a total of 20 weeks of 80 mg/kg/wk NS-065/NCNP-01. Additional patients may be randomized in either treatment group.

3.2.3. Potential Design Modifications Due to Toxicities

Dosing will continue through completion of the high dose cohort or until dose limiting toxicities occur. If dose limiting toxicities that are at least possibly related to the study intervention occur in at least 2 patients at any time, consideration will be given to reduce the dose. If those circumstances occur, any further enrollment in this study will occur at the reduced dose and the dose will be reduced accordingly for current, active patients. Decisions regarding dose reduction for an entire cohort will be made by the Study Chair, Medical Monitor and DSMB in consultation with the Sponsor.

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 from Figure 4 will occur in consultation with the Study Chair, Medical Monitor, Sponsor and the DSMB, depending on the timing and nature of the toxicities. The overall dose escalation scheme may be modified based on cumulative safety findings during the course of the study.

Once safety data from both cohorts are assessed, the highest observed tolerated dose will be defined as the dose level below the dose which the Study Chair, Medical Monitor, Sponsor and DSMB determine to be an unacceptable risk to patients. It is possible that the highest observed dose in this study, 80 mg/kg may be considered a safe dose, but is not the maximum tolerated dose. Higher dosing will not be pursued in this study; therefore, a maximum tolerated dose may not be observed or needed in this study.

Patients who have completed this study may choose to receive NS-065/NCNP-01 in an open-label extension study (NS-065/NCNP-01-202). Patients will receive the same dose in the extension study as they received in the current study NS-065/NCNP-01-201 to ensure continuity of care and balance this with ongoing safety considerations from the emerging NS-065/NCNP-01 clinical data.

Patients who choose not to enter the extension study will be followed for 30 days to assess safety parameters.

3.3. Study Duration and Dates

The expected study duration for each patient is approximately 32 weeks. The screening period will last approximately 2-4 weeks. For both cohorts, Period 1 is a four (4) week double-blinded period in which participants will be randomized in a 3:1 ratio to receive either active study drug or matching placebo. For both cohorts, Period 2 is a twenty (20) week treatment period in which all patients will receive open-label study drug. The dose level of study drug a patient receives is based on the cohort in which he is enrolled. The follow-up phase is 30 days for patients who do not enter the extension study.

4. STUDY POPULATION SELECTION

4.1. Study Population

Approximately 16 patients (4-<10 years of age) with DMD amenable to exon 53 skipping that meet the eligibility criteria below will be enrolled.

4.2. Inclusion Criteria

1. Patient's parent or legal guardian has provided written informed consent/HIPAA authorization prior to any study-related procedures and patient has provided assent appropriate for his age and developmental status;
2. Patient has a confirmed diagnosis of DMD defined as:
    a. Patient is male with clinical signs compatible with DMD; and
    b. Patient has a confirmed DMD mutation(s) in the dystrophin gene that is amenable to skipping of exon 53 to restore the dystrophin mRNA reading frame including determination of unambiguous defined exon boundaries (using techniques such as Multiplex Ligation-dependent Probe Amplification (MLPA), Comparative Genomic Hybridization (CGH) array or other techniques with similar capability);
3. Patient is ≥ 4 years at time of consent and <10 years of age at time of first infusion in the study;
4. Patient is able to walk independently without assistive devices;

5. Patient is able to complete the time to stand (TTSTAND), time to run/walk 10 meters (TTRW) and time to climb 4 stairs (TTCLIMB) assessments as determined by the Clinical Evaluator (CE) at Screening;
6. Clinical safety laboratory test results (refer to Table 2) are within the normal range at the Screening Visit, or if abnormal, are not clinically significant, in the opinion of the Investigator;
7. Patient and parent/guardian are willing and able to comply with scheduled visits, investigational product administration plan, and study procedures;
8. Patient must be on a stable dose of glucocorticoid for at least 3 months prior to study entry, and is expected to remain on the stable dose of GC treatment for the duration of the study.

4.3. Exclusion Criteria

1. Patient has had an acute illness within 4 weeks prior to the first dose of study medication, in the opinion of the Investigator;
2. Patient has evidence of symptomatic cardiomyopathy. [Note: Asymptomatic cardiac abnormality on investigation would not be exclusionary];
3. Patient has a severe allergy or hypersensitivity to study medication;
4. Patient has severe behavioral or cognitive problems that preclude participation in the study, in the opinion of the Investigator;
5. Patient has previous or ongoing medical condition, medical history, physical findings or laboratory abnormalities that could affect safety, make it unlikely that treatment and follow-up will be correctly completed or impair the assessment of study results, in the opinion of the Investigator;
6. Patient is taking any other investigational drug currently or within 3 months prior to the start of study treatment;
7. Patient has had surgery within the 3 months prior to the first anticipated administration of study medication or surgery is planned for anytime during the duration of the study;
8. Patient has previously participated in this study or any other study during which NS-065/NCNP-01 was administered;

9. Patient has positive test results for hepatitis B antigen, hepatitis C antibody or HIV antibody at screening.

Note: Any parameter/test may be repeated at the Investigator's discretion during Screening and/or Day -1 to determine sustainability and reproducibility. In addition, patients may be rescreened if ineligible due to a transient condition which would prevent the patient from participating, such as an upper respiratory tract infection.

5. INVESTIGATIONAL PRODUCT

5.1. Description of NS-065/NCNP-01 and Placebo

Investigational product is provided in 10 mL glass vials for dilution and intravenous administration.

- NS-065/NCNP-01: 10 mL glass vial containing 25 mg/mL of drug substance solution in saline
- Placebo: 10 mL glass vial of saline

Description:

- NS-065/NCNP-01: Clear, colorless to pale yellow solution
- Placebo: Matching in clarity and color to NS-065/NCNP-01

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 by Xerimis in Moorestown, NJ. Additional details for ordering the investigational product can be found in the IPIM and the Manual of Operations.

5.2. Dispensing Investigational Product

For the first 4 weeks of participation (Period 1), patients will be randomized to active drug (low dose or high dose) or placebo. Patients will receive IV infusions of NS-065/NCNP-01 Injection or matching placebo administered once weekly. For the subsequent 20 weeks of participation (Period 2), patients will receive active drug at either low dose (40 mg/kg/wk) or high dose (80 mg/kg/wk), depending on which cohort they have been enrolled in. Patients will receive IV infusions of NS-065/NCNP-01 Injection administered once weekly.

Investigational product will be prepared in accordance with the IPIM by the study site pharmacy and administered by IV infusion over a 1-hour period.

5.3. Instructions for Administration of Investigational product

Prepared investigational product (diluted solution) is administered intravenously within 5 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

Both the investigational product and the placebo will be of equal volume and equal appearance, thus maintaining the blind. Labeling during the first 4 weeks of treatment (Period 1) will not indicate whether investigational product is active drug or placebo. Labels for the unblinded portion (Period 2) will indicate that the investigational product is NS-065/NCNP-01.

All site investigators, coordinators, and pharmacists as well as the CRO will remain blinded to treatment assignment.

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 +/- 2-day window. Weekly study drug treatments for this study should be calculated from the 1st infusion, 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 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 an 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 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. 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 glucocorticoid steroids and other pharmacological medications including over the counter medications, herbal remedies, supplements and vitamins used within 3 months before signing the informed consent form (ICF) will be recorded in source documents and in the electronic case report form (eCRF). All medications taken throughout the study will be recorded in source documents and 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 within 1 year of signing the ICF 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

Investigators are reminded to minimize concomitant medication use or changes to glucocorticoid steroid use unless necessary for medical management. Any other experimental/investigational products are prohibited from 3 months prior to first dose (exclusion criterion) throughout participation in this 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:

- Pre-treatment Screening Phase: From execution of the Informed Consent/HIPAA authorization/Assent until Day 1 (1st Infusion).
- Treatment Phase: The 24-week interval starting with administration of the first dose of study medication or placebo on Day 1 and continuing through the time of administration of the final dose of study medication on Week 24 and completion of Week 24 safety, PK, and clinical efficacy assessments.
    - Period 1: The 4-week interval starting with the administration of the first dose of study medication or placebo on Day 1 and continuing through the time of administration of the Week 4 dose of study medication and placebo and completion of Week 4 safety, PK, PD and clinical efficacy assessments.
    - Period 2: The 20-week interval starting with administration of open-label dose of study medication on Week 5 and continuing through the time of administration of study medication on Week 24 and completion of Week 24 safety, PK, and clinical efficacy assessments.
- Post-treatment Phase: The 30-day interval (including Week 25) beginning after completion of the 24-week Treatment Phase and ending after a final phone call for

collection of any information about adverse events and concomitant medications for those patients who do not elect to enroll in the open-label extension study (NS-065/NCNP-01-202) that will follow this study. Patients who proceed into the extension study will continue to be followed and therefore will not require a follow-up phone call.

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Table 1. Schedule of Study Assessments

	Pre-Treatment Phase		Treatment Phase																							Post Treatment Phase		
Week	Screening Visit	Pre-Infusion Visit	1st infusion	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	Follow-Up Call	ET
	Day-21 (+/- 7day window)	Day -7 (+6 day window)	Day 1	± 2 day window for each weekly visit																							±3 days	30 days (+3 days) from last dose	
Pre-Study Assessments																													
Consent/Assent	X																												
Inclusion/Exclusion	X	X																											
Genetic Confirmation	X																												
Antigen and antibody testing[1]	X																												
Demographics	X																												
Medical and Surgical History	X																												
Medication and Treatment History	X	X	X																										
Investigative Drug																													
Drug 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			
Safety Assessments																													
Height, & Weight	X	X	X				X				X				X				X				X				X		X
Vitals[11]	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				X				X				X				X		X
Medical, Surgical, Medication, and Treatment 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
Hematology[2]	X				X		X		X		X				X				X				X				X		X
Chemistry[3]	X				X		X		X		X				X				X				X				X		X
Urinalysis (Random Urine)[4]	X				X		X		X		X				X				X				X				X		X
Urinalysis (24hr pooled Urine)[5]	X		X																							X			X
12-lead ECG	X		X												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[6]			X												X											X			X
anti-dystrophin antibody			X												X											X			X
anti-NS-065/NCNP-01 antibody			X				X								X											X			X
Efficacy Asssessments																													
Muscle biopsy (for dystrophin)[7]		X																									X		X
Function and Strength[8]	X	X[10]													X												X[10]		X[10]
Pharmacokinetics and Pharmacodynamics																													
PK (Blood)[9]			X				X								X											X			X
Serum PD Biomarker	X														X												X		X

Notes:

1. Antigen and antibody testing: HBs antigen, HCV antibody, HIV antibody
2. Hematology: RBC, Hb, Ht, Ret, MCV, MCH, MCHC, WBC, PLT, WBC Differential Fibrinogen, aPTT, PT-INR
3. 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
4. Urinalysis (Random Urine): Urine glucose, Urine blood, Urine urobilinogen, Urine specific gravity, Urine osmolality, Urinary sediment (RBC, WBC, Cast), Urine protein (benzothonium chloride method), Albumin in urine, NAG in urine, α1-microglobin in urine, Urine Cr
5. 24hr pooled Urine : Urine protein (benzothonium chloride method) , Albumin in urine, NAG in urine, α1-microglobin in urine, Urine Cr, Urine Na, Urine K, Urine Cl, Urine IP, Uric acid in urine, β2-microglobin in urine; 24hr pooled sample also used for PK Urine at 1st infusion and Wk24.
6. Cytokine (IL-6, TNF-α, MCP-1). Sampling time points from time of infusion start: D1 and Wk24: Pre-dose, 9hr (±30min) and 24hr (±1hr); Wk 13: Pre-dose and 9 hr (±30min).
7. For the early termination subject, the muscle biopsy is performed if acceptable.
8. 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
9. Sampling time points from time of infusion start: D1 and Wk 24: Pre-dose, 0.5hr, 1hr (at end of infusion), 1.25hr, 2hr, 3hr, 5hr, 9hr, and 24hr; Wk 5 and 13: Pre-dose, 2hr, and 9hr; ET: Clinical Judgement.
10. Function test can be performed one day prior to mucle biopsy
11. Sampling time points from time of infusion start: Pre-dose, 1hr (at end of infusion), 2hrs, and 6hrs (if 2hr collection shows significant change from pre-dose).

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-01-201 participation begins once written informed consent is obtained from the parent/legal guardian for a patient participant before any study-specific procedures are performed.

Following the signing of the written ICF/Assent Form, patients will be assigned a unique, site-specific, 6-digit patient identification number in sequential order of screening into the study. The patient identification number will be assigned by the site at the time of submission of the de-identified genetic test report to the central genetic counselor to confirm that the patient meets the genetic diagnostic eligibility criteria. If the de-identified genetic test report is submitted to the central genetic counselor prior to signing of the ICF (only if acceptable per local IRB/REB) then a screening number will be assigned to the potential patient prior to the signing of the ICF and prior to study participation.

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. Patient identification numbers assigned to patients who fail screening may not be used again.

Each site principal investigator (PI) will keep a Patient Identification log relating the names of the patients to their patient identification numbers to permit efficient verification of patient files, when required.

Patients who have failed screening may be rescreened. If rescreening is more than 90 days since a current ICF/Assent form has been signed and/or the consent form has been modified from their original consent, patients should be re-consented prior to rescreening procedures.

7.4. Genetic Confirmation of Diagnosis

As part of the screening assessments the central genetic counselor will review the de-identified genetic report to confirm the patient's DMD diagnosis and presence of a mutation that is eligible for skipping of exon 53. This assessment and review of past genetic testing is not associated with any risks to the patient. The date of diagnosis, method of diagnosis, diagnosis results will be documented in source documents and captured in the relevant eCRF. If the patient also previously underwent a muscle biopsy as part of their clinical diagnosis of DMD relevant information from the biopsy report such as date of muscle biopsy, muscle biopsy results, and methods will be documented in source documents and captured in the relevant eCRF.

7.5. Demographics

Demographics will be collected during the screening phase. The following information will be collected: date of birth, race, ethnicity, and hand dominance and documented in source documents and captured in the relevant eCRF.

7.6. Medical History

Patient medical, surgical, medication and treatment history will be collected during the screening phase and reviewed throughout the study. The PI or designee will obtain detailed information regarding all past medical and surgical events. The dates and descriptions of past events will be documented in source documents and captured in the relevant eCRF.

7.7. Weight and Height

Standing 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 patient's legs should be kept as close as possible and the patient's heels should be placed back as close to the wall as possible. Patient may hold on to an object to facilitate balance. If a patient 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 7.13.3.7). 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.

7.8. 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, 1 hour (+10 mins) (at end 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 measured again at 6 hours (+/- 5 mins) after initiation of infusion.

Vital signs will include the following:

- Systolic blood pressure,

- Diastolic blood pressure,
- Heart rate,
- Respiratory rate, and
- 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.

7.9. Physical and Neurological Examination

The physical examination will be performed at the visits specified in the schedule of study assessments (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. Significant findings prior to the start of investigational product will be recorded on the Medical History/Current Medical Conditions page of the eCRF. 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.

7.10. 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 local cardiologist interpretation. ECG results will be recorded in the eCRF.

7.12. Clinical Laboratory Tests

Clinical laboratory assessments will be performed at visits specified in the study assessment 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, urinalysis, cytokines, anti-dystrophin antibody, anti-NS-065/NCNP-01 antibody, and viral antigen and antibody testing.

Laboratory safety assessments will be performed at the visits specified in the Schedule of Assessments (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 Table 2.

Table 2. Clinical Laboratory Tests

Hematology, Chemistry and Urinalysis – 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

- Urinalysis
  - Random urinalysis includes:
    - Urine glucose
    - Urine blood
    - Urine urobilinogen
    - Urine specific gravity
    - Urine osmolality
    - Urinary sediment (RBC, WBC, Cast),
    - Urine protein (benzethonium chloride method)
    - Urine microalbumin
    - Urine N-acetyl-beta-D-glucosaminidase
    - Urine α1-microglobulin
    - Urine Cr
  - 24-hour pooled urine includes: **(not included in safety labs for eligibility assessment)**
    - Urine protein (benzethonium chloride method)
    - Urine microalbumin
    - Urine N-acetyl-beta-D-glucosaminidase
    - Urine α1-microglobulin
    - Urine Cr
    - Urine Na
    - Urine K
    - Urine Cl
    - Urine IP
    - Urine uric acid
    - Urine β2-microglobulin in urine
    - Urine PK

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: interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1). Blood will be drawn from patients at pre-dose, 9 hr (+/- 30 mins) and 24 hr (+/-1 hr) after initiation of infusion at Day 1 and Week 24 and at pre-dose and 9 hr (+/- 30 mins) after initiation of infusion at Week 13.

7.12.3. Anti-Dystrophin Antibody

Anti-dystrophin 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).

7.12.4. 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).

7.12.5. Antigen and Antibody Testing

Antigen and antibody testing will be performed during the screening assessment. The following tests will be performed: hepatitis B antigen, hepatitis C antibody, and HIV antibody.

7.13. Pharmacodynamics and Efficacy Assessments

7.13.1. Muscle Biopsy for Dystrophin Pharmacodynamic Analysis

Muscle tissue will be obtained from the bicep muscle by a trained surgeon. Kits for handling and transport of muscle biopsy samples will be provided by the central lab. Procedures for processing, storage and handling of muscle biopsy samples will be specified in the laboratory manual.

7.13.1.1. Dystrophin Measurements

The ability of NS-065/NCNP-01 to engage the dystrophin RNA target and modulate RNA splicing and dystrophin protein production will be assessed by four complementary laboratory methods (1 RNA splicing measure, 3 dystrophin protein measures). The alteration of mRNA splicing will be measured by RT-PCR of dystrophin mRNA transcripts from a patient muscle biopsy, before and after drug treatment. The production of dystrophin protein will be measured by immunoblot (primary endpoint), immunofluorescence staining methods, and stable isotope mass spectrometry methods.

7.13.1.2. Immunoblot (Western blot) (Primary Efficacy Endpoint):

The primary biochemical outcome measure is measurement of drug-induced increase in dystrophin production by Immunoblot (Western blot). Dystrophin immunoblot uses solubilized muscle biopsy cryosections, with proteins fractionated by molecular weight using gel electrophoresis (SDS-PAGE), electroblotting to nitrocellulose, then incubation of nitrocellulose with antibodies to detect dystrophin protein. The immunoblot signal for dystrophin from a patient's biopsy is then compared to the signal of a standard curve of dystrophin on the same gel (mixed DMD and normal controls). This provides a semi-quantitative assessment of dystrophin content in the muscle.

7.13.1.3. RT-PCR, Immunofluorescence Staining, and Mass Spectrometry (MS) (Secondary Endpoints):

RT-PCR measures altered splicing of the dystrophin RNA. In this method, RNA is isolated from the frozen muscle biopsy, reverse transcribed to cDNA, and PCR primers designed flanking the exon 53 site on the dystrophin mRNA. RT-PCR bands corresponding to specific versions of the spliced dystrophin mRNA are visualized by gel electrophoresis, and the amount of different mRNA isoforms compared. If the drug successfully engages the RNA target, then exon 53 is excluded from the resulting mRNA transcripts.

Immunofluorescence staining is a semi-quantitative assessment of dystrophin localization within muscle fibers, and the degree to which muscle fibers show increased dystrophin compared to pre-treatment biopsies. Dystrophin is co-stained with a control protein (laminin alpha 2) that labels all myofibers. On separate slides, dystrophin is co-stained with alpha sarcoglycan to measure re-constitution of the dystrophin-associated membrane cytoskeleton.

Mass spectrometry (MS) measures drug-induced increases in dystrophin production by comparison to control dystrophin labeled with stable isotopes. In this method, a reference control sample is produced from human myotubes grown in vitro in stable isotope amino acids. This leads to the control sample having dystrophin and other proteins labeled with stable isotopes, and thus distinguishable from the experimental test sample from the muscle biopsies of the study patients (not labeled with stable isotope). To measure the dystrophin protein content of the patient muscle biopsy, cryosections of the muscle are solubilized in detergent solutions, then mixed with detergent solutions of the control myotubes. The combined solutions are fractionated

by molecular weight using SDS-PAGE, tryptic peptides extracted and analyzed by MS, where individual peptides of dystrophin, as well as additional reference proteins, are analyzed and compared. This leads to a quantitative assessment of dystrophin protein content in the muscle biopsy.

The dystrophin content of skeletal muscle biopsy prior to treatment with NS-065/NCNP-01 will be compared to dystrophin content after NS-065/NCNP-01 treatment. Increased levels of dystrophin protein will be reflective of engagement of NS-065/NCNP-01 with the RNA drug target, alteration of splicing, and production of de novo dystrophin in patient muscle (pharmacodynamic biomarker).

7.13.1.4. Serum Pharmacodynamic Biomarkers:

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

7.13.2. Function and Strength

All function and strength testing will be performed by a trained site 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.

7.13.2.1. Time to stand (TTSTAND)

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 7.13.3.4). 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 5-point rating scale of how the patient attains the standing position will be documented in source documents and captured in the relevant eCRF.

7.13.2.2. 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 7.13.3.4).

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.

7.13.2.3. 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 7.13.3.4). 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.

7.13.2.4. 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.

7.13.2.5. 6 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 25m 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.

7.13.2.6. 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)

7.13.2.7. 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.

7.14. Pharmacokinetic Assessments

7.14.1. Collection and Assessment of Pharmacokinetic Samples

Pharmacokinetics assessments will be performed 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 1 hour to complete. Blood will be drawn from patients for PK analysis at the following sampling times:

- Day 1 (first dose):
  - Pre-dose (- 30 mins)
  - 0.5 hour (+/- 5 mins)
  - 1 hour (+/- 5 mins) (Should be drawn immediately after completion of infusion)
  - 1 hour and 15 mins (+/- 5 mins) (Should be drawn at 15 minutes after completion of infusion) All efforts will be made to complete infusion over 1 hour and draw blood as close to this scheduled time point as possible.
  - 2 hour (+/- 5 mins)
  - 3 hour (+/- 5 mins)
  - 5 hour (+/- 5 mins)
  - 9 hour (+/- 5 mins)
  - 24 hour (+/- 30 mins)
- Week 5:
  - Pre-dose (- 30 mins)
  - 2 hour (+/- 5 mins)
  - 9 hour (+/- 5 mins)
- Week 13:
  - Pre-dose (- 30 mins)
  - 2 hour (+/- 5 mins)
  - 9 hour (+/- 5 mins)
- Week 24:
  - Pre-dose (- 30 mins)
  - 0.5 hour (+/- 5mins)
  - 1 hour (+/- 5 mins) (Should be drawn immediately after completion of infusion)
  - 1 hour and 15 minute (+/- 5 mins) (Should be drawn at 15 minutes after completion of infusion) All efforts will be made to complete infusion over 1 hour and draw blood as close to this scheduled time point as possible.
  - 2 hour (+/- 5 mins)
  - 3 hour (+/- 5 mins)
  - 5 hour (+/- 5 mins)
  - 9 hour (+/- 5 mins)
  - 24 hour (+/- 30 mins)

The 24-hr pooled urine will be collected for PK analysis on Day 1, and Week 24. Additional 24-hour urine sampling may be completed for safety assessments to monitor potential nephritic events. Any additional 24-hour urine samples will be identified as unscheduled and included in planned analyses by date and time or origin of the samples.

Appropriate PK samples will be collected and processed by a trained member of the study team for shipment to the central laboratory who will forward to BML, Inc. (Japan) for analysis. This assessment is associated with the usual risks a blood draw which include pain, bruise at the point where the blood is taken, redness 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. Procedures for the collection, handling, and shipping of laboratory samples will be specified in the Laboratory Manual.

7.14.2. Shipment of Pharmacokinetic Samples

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

7.14.3. 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, PK and efficacy. Total whole blood volume collected over the study duration is not to exceed 176.9 mL for each patient. A breakdown of total volume of blood collected is summarized in Table 3.

Table 3. Blood Sample Number and Volume

	Visit												Total mL of Blood
Test	Screening	Day 1	Week 3	Week 5	Week 7	Week 9	Week 13	Week 17	Week 21	Week 24	Week 25	ET[d]	
Safety	10.6		10.6	10.6	10.6	10.6	10.6	10.6	10.6		10.6	10.6	95.4
Antigen, antibody and cytokine	10	4					4			4		4	22
PD	1						1				1	1	3
PK[a,e]		18		6			6			18		6	48
Anti-Dystrophin and Anti-NS-065/NCNP-01 (pre-dose)[c]		2.5		1[e]			2.5			2.5		2.5	8.5
	Approximate total volume of blood per patient[b]: 176.9												

a. Blood samples for PK analysis are collected according to the schedule in Section 7.14.1
b. Total volume of blood collected per participant does not include any additional testing that may be required for follow-up of AEs or retesting and follow-up of abnormal laboratory results. Total volume based on maximum expected blood draw from screening through week 25
c. Anti-NS065/NCNP-01 testing only
d. Early termination blood draw volumes based on clinical judgement from PI, study chair and medical monitor
e. For SAEs, additional blood draws for PK and immunogenicity will be based on timing and clinical judgement from PI, study chair and medical monitor

8. STUDY ACTIVITIES

8.1. Pre-treatment Phase

The pre-treatment phase will be comprised a minimum of two visits; a Screening Visit to allow the PI to assess the patient's eligibility, and a Pre-Infusion Visit, to collect a baseline muscle biopsy. The Manual of Operations (MOOP) provides details on order of testing and data collection information.

8.1.1. Screening Day -21(+/- 7)

Potential patients will be screened using the protocol inclusion and exclusion criteria. Screening will include assessments to confirm eligibility (including review to confirm the DMD diagnosis and appropriate mutations). ICF will be obtained prior to any study related procedures being performed.

Screening activities:

- Informed consent
- Confirm DMD diagnosis
- Inclusion/Exclusion
- Demographics
- Medical and Surgical History
- Medication and Treatment History
- Height, Weight, and Vital signs
- Physical and Neurological Exam
- 12-Lead ECG
- Function and Strength:
    - TTSTAND
    - TTCLIMB
    - TTRW
    - 6MWT
    - NSAA
    - QMT
    - Ulnar Length

- Antigen and antibody testing: HBs antigen, HCV antibody, HIV antibody
- Hematology
- Chemistry
- Serum PD biomarker
- 24-hr Pooled Urine
- Urinalysis (Random Urine) at first void of 24-hour urine collection
- AE Review

Patients who fail to meet all entry criteria are considered to be Screen Failures and are not required to return for additional visits (although a patient can be seen at any time for safety reasons). Patients may be rescreened if ineligible due to a transient condition.

8.1.2. Pre-Infusion Visit Day -7 (+6)

- Review of Medical and Surgical History
- Review of Medication and Treatment History (confirmation of concomitant medications and other treatments; any changes will be noted)
- Review of eligibility
- Height, Weight, & Vital Signs
- Physical and Neurological Exam
- Function and Strength (may be completed one day prior to muscle biopsy):
  - TTSTAND
  - TTCLIMB
  - TTRW
  - 6MWT
  - NSAA
  - QMT
- Muscle biopsy (bicep)
- AE Review

8.2. Treatment Phase

8.2.1. Day of First Infusion (Day 1)

On Day 1 the following activities will occur:

- Review of Medical and Surgical History
- Review of Medication and Treatment History (confirmation of concomitant medications and other treatments; any changes will be noted)
- Height, Weight, &Vitals
- Physical and Neurological Exam (to check that recovery of muscle biopsy; if not recovered then delay of Day 1)
- 12-Lead ECG
- Anti-dystrophin antibody (pre-dose)
- Anti-NS-065/NCNP-01 antibody (pre-dose)
- Investigational product administration
- Cytokine
- PK Blood
- 24-hr Pooled Urine: Samples are pooled and collected for 24 hours after start of administration
- AE Review

8.2.2. Week 2

At the Week 2 visit the following activities will occur:

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

8.2.3. Week 3

At the Week 3 visit the following activities will occur:

- Review of Medical, Surgical, Medication, and Treatment History
- AE Review

- Vital Signs
- Physical and Neurological Exam
- Hematology
- Chemistry
- Urinalysis (Random Urine)
- Investigational product administration

8.2.4. Week 4

At the Week 4 visit the following activities will occur:

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

8.2.5. Week 5

At the Week 5 visit the following activities will occur:

- Review of Medical, Surgical, Medication, and Treatment History
- AE Review
- Height, Weight, & Vital Signs
- Physical and Neurological Exam
- Hematology
- Chemistry
- Urinalysis (Random Urine)
- Anti-NS-065/NCNP-01 antibody (pre-dose)
- Investigational product administration
- PK Blood

8.2.6. Week 6

At the Week 6 visit the following activities will occur:

- Review of Medical, Surgical, Medication, and Treatment History
- AE Review

- Vital Signs
- Investigational product administration

8.2.7. Week 7

At the Week 7 visit the following activities will occur:

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

8.2.8. Week 8

At the Week 8 visit the following activities will occur:

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

8.2.9. Week 9

At the Week 9 visit the following activities will occur:

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

8.2.10. Week 10

At the Week 10 visit the following activities will occur:

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

8.2.11. Week 11

At the Week 11 visit the following activities will occur:

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

8.2.12. Week 12

At the Week 12 visit the following activities will occur:

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

8.2.13. Week 13

At the Week 13 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
    - TTCLIMB

  - TTRW
  - 6MWT
  - NSAA
  - QMT
  - Ulnar Length
- Hematology
- Chemistry
- 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

8.2.14. Week 14

At the Week 14 visit the following activities will occur:

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

8.2.15. Week 15

At the Week 15 visit the following activities will occur:

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

8.2.16. Week 16

At the Week 16 visit the following activities will occur:

- Review of Medical, Surgical, Medication, and Treatment History

- AE Review
- Vital Signs
- Investigational product administration

8.2.17. Week 17

At the Week 17 visit the following activities will occur:

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

8.2.18. Week 18

At the Week 18 visit the following activities will occur:

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

8.2.19. Week 19

At the Week 19 visit the following activities will occur:

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

8.2.20. Week 20

At the Week 20 visit the following activities will occur:

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

8.2.21. Week 21

At the Week 21 visit the following activities will occur:

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

8.2.22. Week 22

At the Week 22 visit the following activities will occur:

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

8.2.23. Week 23

At the Week 23 visit the following activities will occur:

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

8.2.24. Week 24

At Week 24 visit the following activities will occur:

- Review of Medical, Surgical, Medication, and Treatment
- AE Review
- Vital Signs
- 24-hr Pooled Urine: Samples are collected for 24 hours after start of administration
- Cytokine
- Anti-dystrophin antibody (pre-dose)
- Anti-NS-065/NCNP-01 antibody (pre-dose)
- Investigational product administration
- PK Blood

8.3. Post-Treatment Phase

8.3.1. Week 25 or Early Termination Visit

The following activities will occur at the end of the study (7 days (+/- 3 days) after the Week 24 dose) for all patients or for a patient who withdraws early from the study or is withdrawn by the site PI (Early Termination Visit). Note that at Early Termination, the muscle biopsy is at the discretion of the investigator:

- Review of Medical, Surgical, Medication, and Treatment History
- AE Review
- Height, & Weight
- Physical and Neurological Exam
- 12-Lead ECG
- Function and Strength
    - TTSTAND
    - TTCLIMB
    - TTRW
    - 6MWT
    - NSAA
    - QMT

- Ulnar Length

- Hematology
- Chemistry
- Serum PD biomarker
- 24-hr Pooled Urine (Only at Early Termination)
- Cytokine (Only at Early Termination)
- Anti-dystrophin antibody (Only at Early Termination)
- Anti-NS-065/NCNP-01 antibody (Only at Early Termination)
- Urinalysis (Random Urine)
- Muscle Biopsy (bicep) (At Early Termination at the discretion of the investigator)
- PK Blood (Only at Early Termination up to 3 samples collected with timing to be determined based on clinical judgement from site investigator, study chair and medical monitor)

8.3.2. Follow-up Phone Call

Patients who do not enter the extension study (NS-065/NCNP-01-202) should have a phone call conducted with a member of the site study staff, 30 days (+3 days) following the last IP infusion, 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.

8.3.3. 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 in the source documents and the eCRF as an Unscheduled Visit.

8.3.4. 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 Investigator, sponsor, DSMB and/or regulatory authority terminates the study; or
- Any other reason.

A Patient Completion/Discontinuation electronic case report form (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.

8.3.5. 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 8.3, Week 25 or Early Termination Visit will be performed.

8.4. Patient Replacement

If a patient withdraws prior to the Week 4 visit, he will be replaced by enrolling an additional patient to the same dose cohort, assigned to the same treatment group as the patient who withdrew.

If a patient withdraws after more than 4 weeks on the study and prior to 12 weeks on the study, and the total number of patients in each cohort becomes less than eight, an additional patient will be enrolled in the same dose cohort and randomized without regard to the treatment group of the patient who withdrew.

If a patient withdraws after at least 12 weeks of treatment, he will not be replaced.

Detailed procedures for patient replacement will be described in the Manual of Operations.

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 PROCESSESS

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 after completion of study participation.

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 drug brochure or is not listed at the specificity or severity that has been previously observed. During the course of the study, the investigator 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 an SAE need not 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 AEs occurring during the course of the study (starting from signing informed consent to study completion) will be collected on the AE eCRF. Each AE is to be evaluated for duration,

severity, seriousness, and causal relationship to the investigational product. For each AE, 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 AE (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 AE. The question of the relationship of AE 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 ICF 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 database within 24 hours (1 working day) of the Principal Investigator or the clinical site becoming aware of the occurrence.

The Investigator is required to submit SAE reports to the IRB or REB in accordance with local requirements.

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 CRO 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 Development Services is responsible for submitting 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 that require prompt submission to their IRB/REB. The investigator shall report all SAEs occurring within 24 hours after becoming aware via the study database. 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 PharmaLex Development Services.

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 CRO retains the right to request additional information for any patient with ongoing AEs 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 and Management of Abnormal Electrocardiograms

If a clinically significant ECG abnormality occurs that was not present at baseline (screening) 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.

9.12. 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).

9.13. Medical Monitor

The Medical Monitor (MM) 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.

9.14. 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 adverse events 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 after the completion of the first 4 weeks of investigational product is administered for both dose cohorts, and then 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. Summaries of placebo vs NS-065/NCNP-01 will be created by the unblinded statistician and will only be provided to the DSMB, and not to the Study Chair or any other members of the study team.

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 9.2 or higher.

This study includes two cohorts, one at a low dose (n=8) and one at a high dose (n=8). Moreover, within each dose level, 6 patients receive NS-065/NCNP-01 Injection for 24 weeks, while 2 patients receive placebo for the first four infusions and NS-065/NCNP-01 Injection for 20 weeks. The following table provides grouping of the patients in terms of dose cohort assigned and whether they start on NS-065/NCNP-01 or placebo for the first four weeks.

Table 4. Patient groups by dose level (40 vs 80 mg/kg/wk) and initial 4-week treatment (placebo vs NS-065/NCNP-01)

Cohort/Initial Treatment	Placebo	NS-065/NCNP-01	Total
40 mg/kg/wk	Group 1; n=2	Group 2; n=6	n=8
80 mg/kg/wk	Group 3; n=2	Group 4; n=6	n=8
Total	n=4	n=12	n=16

Statistical analyses will occur at the following times for the following groups:

- After the low dose cohort completes the first four weeks of treatment (Groups 1 and 2). Overall safety summaries for all 8 patients will be generated, as well as unblinded summaries for the 6 patients receiving active drug, as well as for the 2 patients on placebo, for the DSMB.
- After the high dose cohort completed the first four weeks of treatment. Overall and unblinded summaries will be generated for the DSMB for the initial 4 weeks of the 4 (Groups 1 and 3) placebo patients versus the 6 low dose patients (Group 2) versus the 6 high dose patients (Group 4).
- 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 and after dystrophin data are received from the second biopsy. 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. A separate report will be generated for the pharmacokinetic analyses.

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

10.2. Determination of Sample Size

Sample size considerations are based on two types of outcomes - categorical types such as AEs, and continuous outcomes such as the % of normal dystrophin measurements and safety laboratory values.

The Table below provides probabilities of observing at least one event in either the placebo group (n=4) or a dose group for the full 24 weeks (n=6) or the full dose group (n=8) or the overall population (n=16) for a range of underlying true probabilities of AEs.

Table 5. Probabilities of observing at least 1 event and at least 2 events based on true underlying toxicity rates

Underlying	Probability of Observing at least 1 event				Probability of Observing at least 2 events			
Event rate	N=4	N=6	N=8	N=16	N=4	N=6	N=8	N=16
10%	0.34	0.47	0.57	0.81	0.05	0.11	0.19	0.49
15%	0.48	0.62	0.73	0.93	0.11	0.22	0.34	0.72
20%	0.59	0.74	0.83	0.97	0.18	0.34	0.50	0.86
25%	0.68	0.82	0.90	0.99	0.26	0.47	0.63	0.94
30%	0.76	0.88	0.94	>0.99	0.35	0.58	0.74	0.97
35%	0.82	0.92	0.97	>0.99	0.44	0.68	0.83	0.99
40%	0.87	0.95	0.98	>0.99	0.52	0.77	0.90	>0.99

The table shows that the study is large enough to observe at least once any AE with an underlying rate of at least 10% with >80% probability. Thus, most reasonably common AEs will be observed even in this small study and will provide information for future studies. Rare events, with underlying rates of <10%, are less likely to be observed in this small study. This sample size consideration addresses the primary objective of safety and tolerability based on the primary safety outcome of AEs,

The second sample size considerations are for the continuous outcomes of both primary safety (laboratory markers; 2nd primary safety outcome) and main efficacy (% of normal dystrophin measurements). Table 6 below shows the detectable differences in the laboratory outcomes over the first 4 weeks between the dose groups and placebo.

Table 6. Detectable differences in standard deviations (SDs) units in laboratory values between NS-065/NCNP-01 and placebo. A two-sided Type I error of 0.05 is assumed.

	40 or 80 mg/kg group vs combined placebo	40 or 80 mg/kg group vs combined placebo	40 and 80 mg/kg group vs combined placebo	40 and 80 mg/kg group vs combined placebo
NS-065/NCNP-01 (n)	6	6	12	12
Placebo (n)	4	4	4	4
Power (%)	80	90	80	90
Detectable SD	2.1	2.4	1.7	2.0

The table shows that at the end of the study, there will be 80% probability of detecting any difference of 2.1 SDs or more in a laboratory parameter between the combined placebo group (n=4; Groups 1 and 3) and any of the dose groups (n=6; either Group 2 or Group 4) while controlling for two-sided Type I error of 0.05. The study will have 90% power to detect any difference of 2.4 SDs or more. When comparing the combined treatment groups (n=12; Groups 2 and 4) and combined placebo groups (n=4; Groups 1 and 3), the study will have 80% power to detect differences of 1.75 SDs and 90% power to detect differences of 2.0 SDs under the same assumptions. These calculations apply whether 4-week values are compared or the differences from baseline at 4 weeks are compared Table 7 below shows the detectable differences in SDs between the two doses (for any time point after the first four weeks) with 80% and 90% power.

Table 7. Detectable differences in standard deviations (SDs) units in laboratory values between high dose and low dose of NS-065/NCNP-01. A two-sided Type I error of 0.05 is assumed.

NS-065/NCNP-01 dose	80% power	90% power
40 mg/kg (n)	8	8
80 mg/kg (n)	8	8
Power (%)	80	90
Detectable SD	1.5	1.7

It is not known what the SD of the mean in % normal dystrophin measured by MS in this age group of DMD boys is. However, a sample size of 8 in one cohort is large enough to detect an increase from no detectable dystrophin to 5% of normal with 80% power, assuming that the SD of the difference from baseline in % normal dystrophin is 4% in boys with DMD, and controlling for a Type I error of 0.05. Even if the SD for change from baseline is as high as 6.5%, both cohorts combined (n=16) would provide 80% power to detect an improvement of 5% of normal dystrophin, while controlling for a two-sided Type I error of 0.05.

10.3. Analysis Populations

The safety population will consist of all randomized patients who received at least 1 dose of investigational product. Patients will be analyzed as treated. This will be the primary analysis population for the evaluation of exposure and safety.

The modified Intent-to-Treat (mITT) population will consist of all randomized patients who received at least 1 dose of investigational product and have a baseline assessment and at least 1 post baseline efficacy assessment. Patients will be analyzed as randomized. This will be the primary analysis population for the evaluation of efficacy.

The PK concentration population will consist of all randomized patients who received at least 1 dose of investigational product and have at least 1 plasma concentration value for investigational product.

10.4. Demographics and Baseline Characteristics

For analyses of baseline data, patients will be grouped in two different ways and summaries provided accordingly.

Grouping A will be the 4 patients who received placebo for 4 weeks before switching to NS-065/NCNP-01 (Groups 1 and 3 in Table 4), *vs.* the 6 low dose patients who received the low dose for 24 weeks (Group 2 in Table 4), *vs.* the 6 high dose patients who received the high dose for 24 weeks (Group 4 in Table 4). These summaries are for the purpose of later summarizing change in safety parameters for the first four weeks.

Grouping B of the baseline data is by the two dose groups (Groups 1 and 2 combined vs Groups 3 and 4 combined) and is applicable for all longer than 4 weeks safety assessments, and all efficacy assessments.

Summaries of patient demographics (age, race, and ethnicity) and baseline safety characteristics (anthropometrics, vital signs, physical examination, hematology, chemistry, urinalysis, ECG and antibodies) will be done with both groupings of data. Summaries of all efficacy parameters will only be done by the groups defined by Grouping B.

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 the primary objective of the study.

10.5.1. Anthropometrics, Vital Signs, Laboratory Assessments, and ECG

Anthropometrics, vital signs, hematology, chemistry, urinalysis, and ECG results will be summarized by dose level over time using descriptive statistics for continuous outcomes. Data collected during Period 1will be summarized by Grouping A and compared to the baseline of Grouping A. For all subsequent data collection points, the safety assessments will be summarized by Grouping B and compared to the baseline of Grouping B for any changes from baseline. Further, all lab abnormalities will be listed.

Antibodies will be summarized by Grouping A for Period 1 data, and by Grouping B for Period 2 data.

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.

Treatment-emergent AEs (TEAEs) will be summarized for Period 1 by Grouping A and for Period 2 by Grouping B. 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. 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 within Period 1, within Period 2 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. 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.

10.6. Efficacy Endpoints

All efficacy analyses will use either Grouping B or the overall mITT group combined. In addition, for secondary objective 2, a historical control group from the CINRG DNHS will be used.

10.6.1. Efficacy Objective - dystrophin production

Dystrophin production will be measured prior to the first infusion and after all 24 IV infusions of NS-065/NCNP-01 Injection are complete. Results will be summarized and normality assessed. If needed, a transformation to achieve normality will be performed. Within patient changes in %

normal dystrophin production (possibly transformed) will be tested using a paired t-test within dose group and with both dose groups combined. Also, a two sample t-test to compare change across the two dose levels will be performed. The same approach will be used for the dystrophin protein measurement by Western blot, mass spectrometry and immunofluorescence staining, and by RT-PCR for mRNA analysis.

10.6.2. Secondary Objectives – timed function tests and muscle strength

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

The TTSTAND, TTCLIMB, TTRW, 6MWT, NSAA, and QMT assessments will be performed at Screening Visit, Pre-Infusion Visit, Week 13, Week 25 and at early termination. The actual values and change from baseline (Pre-Infusion Visit) values will be summarized descriptively at each visit. The TTSTAND, TTCLIMB, and TTRW 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 transformation to achieve normality will be performed for these assessments. Within patient changes (possibly transformed) will be tested using a paired difference t-test within each dose group and also with both dose groups combined. A two sample t-test to compare change across the two dose levels will also be presented.

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.

The secondary efficacy outcome measures (TTSTAND, TTCLIMB, TTRW, 6MWT, NSAA and QMT results) will be compared between the NS-065/NCNP-01 patients and the CINRG DNHS

patients using mixed-effects linear models. The TTSTAND, TTCLIMB, and TTRW times to perform the test will 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.)

10.7. Pharmacokinetic Endpoints and Analysis

All PK analyses will be performed using the PK Population. Pharmacokinetic parameters will be estimated with non-compartmental methods for samples obtained on Day 1 (1st dose) and Week 24. Pharmacokinetic parameters will be derived using model-independent methods as implemented in WinNonlin® (version 6.4) and will be based on plasma concentrations of NS-065/NCNP-01from those subjects who have received a dose of investigational product and have evaluable plasma concentration-time profiles.

Pharmacokinetic parameters will be calculated for each subject for each treatment/analyte as follows:

- C_{max} (Maximum plasma concentration)
- T_{max} (Time of maximum plasma concentration)
- C_{last} (Last quantifiable plasma concentration)
- T_{last} (Time of Last quantifiable plasma concentration)
- $t_{1/2z}$ (Elimination half-life)
- λ_z (Terminal phase rate constant)
- Vd_{ss} (Volume of Distribution)
- $AUC_{0\text{-}24}$ (Area under the plasma concentration-time curve from time zero to 24 hours post-dosing)
- $AUC_{0\text{-}last}$ (Area under the plasma concentration-time curve from time zero to the last quantifiable concentration (C_{last}) post-dosing)
- AUC_{inf} (Area under the plasma concentration-time curve from time zero to infinity)

Individual elapsed sampling times will be used in the pharmacokinetic analysis. C_{max}, T_{max}, C_{last}, and T_{last} will be obtained directly from the experimental observations. $AUC_{0\text{-}24}$ will be calculated using the linear trapezoidal rule.

AUC_{inf} will be calculated according to the following equation:

$$AUC_{inf} = AUC_{0\text{-}last} + \left(\frac{C_{last}}{\lambda_z}\right)$$ where C_{last} is the last quantifiable concentration.

For the purpose of calculating AUC_{0-24}, when two consecutive plasma concentrations below the lower limit of quantification (LLOQ) are encountered after T_{max}, all subsequent values will be excluded from the analysis. When embedded missing values occur, they will be excluded from the analysis. Any quantifiable concentrations at pre-dose will be set to zero.

The exponential rate constant of the terminal-phase, λz, will be estimated by linear regression of the log concentration-time data associated with the terminal phase of the plasma concentration-time profile. The number of data points included in the regression will be determined by visual inspection. A minimum of 3 data points in the terminal phase, excluding C_{max}, will be required to estimate λz.

The terminal half-life, t½z, will be calculated as:

$$t_{½z} = \frac{Ln(2)}{\lambda_z}$$

Summary statistics for continuous pharmacokinetic variables will include n, mean, standard deviation, coefficient of variation (%CV), median, minimum, and maximum. The geometric mean and standard error (SE) of the geometric mean will also be computed and reported for pharmacokinetic parameters. T_{max} will be summarized using n, median, minimum, and maximum values. All available concentration-time data will be listed. Derived PK parameters will be listed by treatment.

Linear-Linear and Log-Linear Mean plots and by subject plots will be completed to display plasma concentrations of NS-065/NCNP-01 for each dose administered.

10.8. Interim Analyses

No interim analyses are planned except for the safety analyses at the completion of the first four weeks of the low and high dose treatment groups as described above. In addition, semi-annual reports to the DSMB will be descriptive and focus on safety. There are no interim analyses on any efficacy outcome.

10.9. 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.

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 (GCP) 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 IRBs/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, NSP 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: [redacted]
Email: [redacted]

Medical Monitor
Helmut H. Albrecht, MD
PharmaLex Development Services, LLC
3350 SW 27th Ave, Apt 2203
Miami, FL 33133
Phone: [redacted]
Email: [redacted]

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 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, Investigational New Drug (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 its 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 completed 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.

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.

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 completed 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 completed. 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 completed 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 NS Pharma 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 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.

12. REFERENCE LIST

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