Protocol number: NS101_P1_01

AMENDMENT III

A PHASE 1, RANDOMIZED, PLACEBO-CONTROLLED, DOUBLE-BLIND, SINGLE ASCENDING DOSE STUDY TO ASSESS THE SAFETY, TOLERABILITY, PHARMACOKINETICS, AND PHARMACODYNAMICS OF INTRAVENOUS NS101 INFUSION IN HEALTHY VOLUNTEERS

Contract Research Organization:

Syneos Health Clinique inc.
(« Syneos Health »)

2500, rue Einstein
Québec (Québec)
Canada, G1P 0A2
Tel.: 1-418-527-4000

Sponsor:

Neuracle Science Co., LTD.

Sanhakgwan 702-2, Anam-ro 145
Seongbuk-gu
Seoul
02841
Republic of Korea
Tel.: 82-2-6949-0409

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Protocol Historical File

Version number	Brief description/summary of changes	Date
Final	Version submitted to the Institutional Review Board (IRB).	24-SEP-2021
Amendment I	Draft version dated 25-OCT-2021 was sent to Health Canada for review. Correction of administrative information/typographical errors. Modifications required by Health Canada following a Clinical Information Request included changes and clarifications in the introduction, decision-making process of the Safety Review Committee, assessment of severity of adverse events, and management of infusion-related reactions.	01-NOV-2021
Amendment II	Inclusion of changes from previous administrative letter. Administrative changes and clarifications regarding drug supplies and timing of vital signs measurements. Removal of quantitative electroencephalogram and orthostatic vitals signs measurements, and adjustment of procedures surrounding collection of cerebrospinal fluid. Broadening of exclusion criterion related to allergic reactions. Minor editorial changes.	26-JAN-2022
Amendment III	Please see summary of changes below.	06-APR-2022

Summary of Changes for Amendment III:

Clarifications included in the administrative letter dated 23-FEB-2022, consisting in a correction of a typographical error in the stopping rules, have been incorporated into the currently amended protocol under sections 8.4 Dose Escalation Scheme and 9.2.3.1 Events Within One Dose Level.

In addition, the following changes were included:

Based on emerging interim clinical results in the current study up to Cohort 4 (3 mg/kg), as presented in the Safety Review Committee meetings, as well as available preclinical data, two more cohorts at two further ascending dose levels have been added. As such, following dosing of the 6 initial cohorts, additional Cohorts 7 and 8 have been assigned to receive doses of 24 mg/kg and 48 mg/kg, respectively. The rationale for these changes is included in an accompanying document.

This change impacts the following sections:

- 3. Synopsis of Protocol: Study Design; Subjects; Inclusion/Exclusion Criteria; Study Drug Administration; Study Restrictions; CSF Sample Collection.
- 4. Schedule of Events
- 5.4.2 PK and PD Parameters

- 8. Study Design
- 8.1 Design for Cohorts 1 to 8
- 8.4 Dose Escalation Scheme
- 10.1 Sample Size
- 10.3 Exclusion Criteria
- 11.3 Randomization and Blinding
- 11.6 Study Treatments Administration
- 11.7.5 Other Restrictions
- 11.8.3 Lumbar Puncture for CSF Sampling

Minor editorial changes were also applied.

Sponsor Signature Page

Neuracle Science Co., LTD.

Sanhakgwan 702-2, Anam-ro 145
Seongbuk-gu
Seoul
02841
Republic of Korea
Tel.: 82-2-6949-0409

Sponsor's representative:

_____ _____

Joseph Park
Chief Medical Officer

Date

Investigator Signature Page

I have carefully read this study protocol and agree that it contains all necessary information required to conduct this study. I agree to conduct the study according to this protocol (including any amendments) and in accordance with the clinical site's Standard Operating Procedures (SOPs), ICH Good Clinical Practice (GCP), all other applicable regulations, and the recommendations laid down in the most recent version of the Declaration of Helsinki.

_____ _____

Date

_____

(printed name)

1. Facilities and Responsible Staff

1.1 Clinical Research Facilities

This study will be conducted by Syneos Health at the following facility:

2500, rue Einstein
Québec (Québec), Canada, G1P 0A2
Tel.: 1-418-527-4000

Screening and/or visits may also be performed at the Montréal Syneos Health facility:

5160, boul. Décarie, suite 800
Montréal (Québec), Canada, H3X 2H9
Tel.: 1-514-485-7500

1.2 Biomedical Laboratory Facilities (Clinical Laboratory Assessment)

Biomedical laboratory testing will be performed by the following laboratories:

Safety assessment:

CDL Laboratories
5990 Chemin de la Côte-des-neiges
Montréal (Québec), Canada, H3S 1Z5
Tel.: 1-514-344-8022

Syneos Health
2500, rue Einstein
Québec (Québec), Canada, G1P 0A2
Tel.: 1-418-527-4000

Syneos Health
5160, boul. Décarie, suite 800
Montréal (Québec), Canada, H3X 2H9
Tel.: 1-514-485-7500

If another biomedical laboratory is used, this will be documented and annexed to the protocol.

1.3 Clinical Pharmacology and Regulatory Affairs

Syneos Health
2500, rue Einstein
Québec (Québec), Canada, G1P 0A2
Tel.: 1-418-527-4000

1.4 Bioanalytical Facilities

Syneos Health
2500, rue Einstein
Québec (Québec), Canada, G1P 0A2
Tel.: 1-418-527-4000

Clark V. Williard, B.S.
Executive Director, Mass Spectrometry Operations

Syneos Health
Les Templiers
2400 route des Colles,
06410 Biot, Sophia-Antipolis, France

1.5 Institutional Review Board

Advarra Aurora, Ontario, Canada
372 Hollandview Trail, Suite 300
Aurora, ON L4G 0A5 Canada
Phone: 1-905-727-7989

Table of Contents

2. List of Abbreviations

aa	amino acid
AChEI	acetylcholinesterase enzyme inhibitor
AD	Alzheimer's disease
ADA	anti-drug antibody
ADCC	antibody-dependent cell-mediated cytotoxicity
AE	adverse event
ALP	alkaline phosphatase
ALS	amyotrophic lateral sclerosis
ALT	alanine aminotransferase
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
AUC_{0-24}	area under the concentration-time curve from time zero to time 24 hours
AUC_{0-inf}	area under the concentration-time curve from time zero to infinity (extrapolated)
AUC_{0-t}	area under the concentration-time curve from time zero until the last observed concentration
$AUEC_{0-t}$	area under the PD effect versus time curve from time zero until the last measurable timepoint
BMI	body mass index
BP	blood pressure
CDC	complement-dependent cytotoxicity
CDER	Center for Drug Evaluation and Research
CHMP	Committee for Medicinal Products for Human Use
Cl	apparent body clearance
C_{max}	maximal observed concentration
C_{min}	minimum concentration
CNS	central nervous system
COVID-19	coronavirus disease 2019
CRO	contract research organization
CRS	cytokine release syndrome
CSF	cerebrospinal fluid
CTA	Clinical Trial Application
CTCAE	Common Terminology Criteria for Adverse Events
CV%	coefficient of variation
DMP	data management plan

DRG	dorsal root ganglia
ECG	electrocardiogram
EMA	European Medicines Agency
E_{max}	maximal observed effect
ET	early termination
FAM19A5	Family with sequence similarity 19, member A5
FcRn	neonatal Fc receptor
FDA	Food and Drug Administration
FIH	first-in-human
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HBsAg	Hepatitis B surface antigen
HC	heavy chain
HCV	Hepatitis C virus
HDL	high-density lipoprotein
HED	human equivalent doses
HEENT	head, eyes, ears, nose, and throat
hIgG1	human immunoglobulin G1
HIV	human immunodeficiency virus
HR	heart rate
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
INR	international normalized ratio
IRB	Institutional Review Board
IRR	infusion-related reaction
IV	intravenous
K_{el}	elimination rate constant
LC	light chain
LDL	light-density lipoprotein
LLOQ	lower limit of quantification
LRRC4B	leucine-rich repeat-containing protein 4B
mAb	monoclonal antibody
Max	maximum
MDMA	3,4-methylenedioxymethamphetamine

Min	minimum
MRSD	maximum recommended starting dose
MTD	maximum tolerated dose
NAb	neutralizing antibody
NMDA	N-methyl d-aspartate
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOL	No Objection Letter
OT	oral temperature
PAD	pharmacologically active dose
PCP	phencyclidine
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PO	pulse oximetry
PSP	project specific procedure
PT	prothrombin time
QA	quality assurance
QC	quality control
QT	QT interval
QTcF	Fridericia's corrected QT interval
RR	respiratory rate
SAD	single ascending dose
SAE	serious adverse event
SAP	statistical analysis plan
SAS	statistical analysis system
SCI	spinal cord injury
SD	standard deviation
SOC	System Organ Class
SOP	standard operation procedure
SRC	Safety Review Committee
S-STS	Sheehan Suicidality Tracking Scale
$T_{½\ el}$	terminal elimination half-life
TEAE	treatment-emergent adverse event
TE_{max}	Time of E_{max}
T_{max}	time when the maximal concentration is observed
TLS	tumor lysis syndrome

ULN	upper limit of normal
V_z	apparent volume of distribution
WBC	white blood cell
WT	wild-type

3. Synopsis of Protocol

Project No.:	200242
Sponsor Protocol No.:	NS101_P1_01
Study Title:	A phase 1, randomized, placebo-controlled, double-blind, single ascending dose study to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of intravenous NS101 infusion in healthy volunteers
Study Drug:	NS101 solution for infusion
Study Phase and Type:	Phase 1 – First-in-Human (FIH) Single Ascending Dose (SAD)
Objectives:	Primary objective: To assess the safety and tolerability of NS101 following intravenous (IV) administration of single ascending doses in healthy subjects. Secondary objectives: • To characterize the pharmacokinetic (PK) profile of NS101 in serum and NS101 concentrations in cerebrospinal fluid (CSF) following single IV infusion doses in healthy subjects. • To characterize the pharmacodynamic (PD) profile of NS101 through FAM19A5 in plasma and FAM19A5 concentrations in CSF following single IV infusion doses in healthy subjects. • To evaluate the immunogenicity profile of NS101.
Study Endpoints	Primary endpoints: • Incidence, nature, relatedness, and severity of adverse events (AEs). • Changes in vital signs measurements, physical examination, clinical laboratory test findings, and 12-lead-ECG results. Secondary endpoints: • Serum PK parameters for NS101 will include: AUC_{0-24}, AUC_{0-t}, AUC_{inf}, C_{max}, T_{max}, $T_{½\ el}$, $K_{el,}$ Cl, and V_z. • Concentrations of NS101 in CSF. • Serum to CSF ratio of NS101 concentrations. • Immunogenicity of NS101, measured as the number and percentage of subjects who develop detectable Anti-Drug Antibody (ADA) and Neutralizing Antibody (NAb). Exploratory endpoints: • Effect (E): variation of level of FAM19A5 (Δ FAM19A5) following administration of NS101: o PD: Change in baseline of FAM19A5 levels o PD parameters in plasma: $AUEC_{0-t}$, E_{max}, TE_{max}. o Concentrations of FAM19A5 in CSF.

Study Design:	This will be a single center, Phase 1, double-blind, randomized, placebo-controlled, sequential SAD study, consisting of **8** cohorts (Cohorts 1 to **8**).
Subjects:	Up to **80** healthy adult males, ≥ 18 and ≤ 55 years of age, are planned to be enrolled in the study. The study will consist of 6 cohorts (Cohorts 1 to **8**, 1 cohort per dose level). Each cohort will include 8 subjects (6 subjects receiving a single dose of the study drug NS101 and 2 subjects receiving a single dose of a matching placebo), for a total of **64** subjects planned for evaluation. All cohorts will receive the study drug under fasting conditions. For all cohorts, a staggered dosing schedule will be used and will include 2 sentinel subjects (1 active and 1 placebo) dosed initially (a minimum of 1 hour apart between end of infusion of first sentinel and start of infusion for second sentinel), and the remaining subjects dosed no sooner than 48 hours after the start of sentinel dosing. Cohorts will be dosed sequentially in an ascending design, with at least 14 days between sentinel dosing of each dose level. When safety/tolerability results from the Day 8 visit for the last subject in a cohort (e.g., Cohort 1), as well as available PK data up to 48 hours post-start of infusion, are available, safety parameters will be evaluated by a Safety Review Committee (SRC) to determine the overall safety of that dose level before proceeding to the next dose level. The SRC (composed of at least the Investigator, one medically qualified Sponsor representative and an independent medical monitor) will review the safety, tolerability, and available PK data in order to make decisions regarding continuation of the study at the next prescribed dose level, decreasing the next dose level, repeating a dose level, or not evaluating any additional dose level, based on consideration of the clinical significance of several safety and tolerability parameters. The SRC meeting will be held after at least 7 subjects from the same cohort have been dosed and followed up until the safety and tolerability data have been collected up to 7±1 days post-infusion for non-sentinels (approximately 9±1 days for sentinels) are available. Subjects who withdraw or are withdrawn from the study after dosing, for reasons other than safety and tolerability, may be replaced after consultation between the SRC members. The total number of subjects dosed (including potential replacement subjects) will remain within a maximum of 10 subjects per cohort. Based on the results of this SAD study, more cohorts and/or additional parts, such as multiple ascending dose evaluations, may be performed. These evaluations would occur only following a protocol amendment.
Inclusion/Exclusion Criteria:	**Inclusion Criteria:** 1) Male, non-smokers (no use of tobacco or nicotine products within 6 months prior to screening), ≥18 and ≤55 years of age, with BMI >18.5 and <30.0 kg/m^2 and body weight ≥50.0 kg for males. 2) Healthy as defined by:

	a) the absence of clinically significant illness and surgery within 4 weeks prior to dosing. b) the absence of clinically significant history of neurological, endocrine, cardiovascular, respiratory, hematological, immunological, psychiatric, gastrointestinal, renal, hepatic, and metabolic disease. 3) Subject's score on the Sheehan Suicidality Tracking Scale (S-STS) at screening must be 0. 4) Male subjects who are not vasectomized for at least 3 months prior to dosing, and who are sexually active with a female partner of childbearing potential (childbearing potential females are defined as women that are neither post-menopausal nor surgically sterile) must be willing to use one of the following acceptable contraceptive methods from the study treatment infusion and for 90 days after. a) simultaneous use of condom and hormonal contraceptive used for at least 4 weeks or intrauterine contraceptive device placed for at least 4 weeks for the female partner; b) simultaneous use of condom with spermicide and a diaphragm or cervical cap for the female partner. 5) Male subjects (including men who have had a vasectomy) with a pregnant partner must agree to use a condom from the study treatment infusion and for 90 days after. 6) Male subjects must be willing not to donate sperm for 90 days after study treatment infusion. 7) Capable of consent. **Exclusion Criteria:** 1) Any clinically significant abnormality at physical examination, clinically significant abnormal laboratory test results or positive test for human immunodeficiency virus (HIV), hepatitis B, or hepatitis C found during medical screening. 2) Positive urine drug screen or alcohol breath test at screening or admission. 3) History of asthma, urticaria, anaphylactic reactions, or any other clinically significant allergic reactions to any medication, including biologics, or food, or allergy to any excipient in the formulation. 4) Clinically significant electrocardiogram (ECG) abnormalities (QTcF >450 ms) or vital sign abnormalities (systolic blood pressure [BP] lower than 90 or over 140 mmHg, diastolic BP lower than 50 or over 90 mmHg, heart rate [HR] less than 50 or over 100 bpm) at screening. 5) History of alcohol abuse within 1 year prior to screening or regular use of alcohol within 6 months prior to screening that exceeds 14 units of alcohol per week (1 unit = 150 mL of wine, 360 mL of beer, or 45 mL of 40% alcohol) or 3 to 4 units per day. 6) History of drug abuse within 1 year prior to screening or recreational use of soft drugs (such as marijuana) within 1 month or hard drugs (such as

	cocaine, phencyclidine [PCP], crack, opioid derivatives including heroin, and amphetamine derivatives) within 3 months prior to screening. 7) Participation in a clinical research study involving the administration of an investigational or marketed drug or device within 30 days prior to study treatment infusion, administration of a biological product in the context of a clinical research study within 90 days prior to study treatment infusion, or concomitant participation in an investigational study involving no drug or device administration. 8) Use of medications for the timeframes specified below, with the exception of medications exempted by the Investigator on a case-by-case basis because they are judged unlikely to affect the PK profile of the study drug or subject safety (e.g., topical drug products without significant systemic absorption): a) prescription medications within 14 days prior to study treatment infusion; b) any vaccination, including COVID-19 vaccine, within 14 days prior to study treatment infusion; c) over-the-counter products and natural health products (including herbal remedies such as St. John's wort, homeopathic and traditional medicines, probiotics, food supplements such as vitamins, minerals, amino acids, essential fatty acids, and protein supplements used in sports) within 7 days prior to study treatment infusion, with the exception of the occasional use of acetaminophen (up to 2 g daily); d) depot injection or implant of any drug within 3 months prior to study treatment infusion. 9) Receiving treatment or participation in an organized weight loss program that may cause significant weight gain or loss within 1 month before dosing. 10) Donation of plasma or serum within 7 days prior to dosing. Donation or loss of blood (excluding volume drawn at screening) of 50 mL to 499 mL of blood within 30 days, or more than 499 mL within 56 days prior to study treatment infusion. 11) History of blood dyscrasias, including, but not limited to, thrombocytopenia, thrombocythaemia, or arterial/venous thromboembolic complications. 12) History of lymphatic disorders. 13) History of hypertriglyceridemia. 14) Current or history of malignancy. 15) Significant history of seizures, prior traumatic brain injury, schizophrenia, schizoaffective disorder, or bipolar disorder. 16) Any reason which, in the opinion of the Investigator, would prevent the subject from participating in the study. 17) For subjects in cohorts that will include a lumbar puncture (Cohorts 5 **to 8**): Medical conditions in which a lumbar puncture is contraindicated, including coagulopathy, thrombocytopenia, prior lumbar spinal surgery,

<table>
<tr><td></td><td>or other factor that precludes safe lumbar puncture in the opinion of the Investigator.

18) For subjects in cohorts that will include a lumbar puncture (Cohorts 5 to 8): hypersensitivity to anesthetics, such as lidocaine, or any of its components.</td></tr>
<tr><td>Screening Procedures:</td><td>Demographic data (age, gender, handedness), medical and medication histories, complete physical examination, body measurements (height, weight, and BMI), vital signs (BP, HR, respiratory rate [RR], and oral temperature [OT]), ECG, S-STS, hematology, biochemistry, coagulation, serology (HIV, hepatitis B and C tests), urinalysis, alcohol breath test, and urine drug screen.</td></tr>
<tr><td>Confinement and Visits:</td><td>For all cohorts, subjects will be confined from the day before dosing (Day -1) until after the 48-hour post-start of infusion blood draw. Subjects will come back to the clinical site for all subsequent study assessments on Days 5±1, 8±1, 15±1, 22±2, 29±2, and 60±3 (study exit or early termination [ET]).</td></tr>
<tr><td>Study Treatment and Dosage Form:</td><td>NS101 solution for injection in glass vial developed in 400 mg/16 mL (25 mg/mL) strength, supplied in 20 mL glass injection vials (Neuracle Science Co., LTD., Republic of Korea).

Matching placebo solution: Solution without the active component, composed of the same ingredients as NS101 solution for IV injection. To be supplied in 20 mL glass injection vials. Manufactured by BINEX Co., Ltd., Republic of Korea.</td></tr>
<tr><td>Study Drug Administration:</td><td>For each dose level, the dose to be administered to each subject for a single infusion will be calculated based on subject’s body weight, measured on Day -1.

In each cohort, subjects will receive a single infusion of NS101 or matching placebo under fasting conditions over a period of approximately 60 minutes at the following target dose levels:

<table>
<tr><td>Cohort</td><td>Dose levels of NS101*</td></tr>
<tr><td>1</td><td>1 x 0.25 mg/kg</td></tr>
<tr><td>2</td><td>1 x 0.75 mg/kg</td></tr>
<tr><td>3</td><td>1 x 1.50 mg/kg</td></tr>
<tr><td>4</td><td>1 x 3.0 mg/kg</td></tr>
<tr><td>5</td><td>1 x 6.0 mg/kg</td></tr>
<tr><td>6</td><td>1 x 12.0 mg/kg</td></tr>
<tr><td>7</td><td>1 x 24.0 mg/kg</td></tr>
<tr><td>8</td><td>1 x 48.0 mg/kg</td></tr>
</table>
*Volume of matching placebo will be also determined based on subject weight and NS101 concentration.

As an example, for a human weighing 60 kg assigned to the first dose level of 0.25 mg/kg, the administered dose would be equal to 15 mg.</td></tr>
</table>

	At the end of the infusion, 3 mL of saline solution will be injected to flush the remaining drug into the IV catheter. The end of infusion will be set to the end of the 3 mL flush. For standardization purposes, no food will be allowed from at least 8 hours prior to start of infusion until at least 2 hours after the start of infusion. A light meal will be served more than 2 hours after the start of study drug infusion. Water will be provided *ad libitum* at all times.
Study Restrictions:	Subjects will be asked to refrain from using products that may potentially affect their safety and/or the PK profile of the study drug. Main study restrictions include: • Prescription medication from 14 days prior to dosing and throughout the study; • Any vaccination, including COVID-19 vaccine, from 14 days prior to dosing and throughout the study; • Over-the-counter products from 7 days prior to dosing and throughout the study; • Natural health products from 7 days pre-dose and throughout the study; • Food containing poppy seeds within 24 hours prior to admission; • Food or beverages containing xanthine derivatives or xanthine-related compounds or energy drinks from 48 hours before dosing and during confinement; • Alcohol-based products from 24 hours prior to admission, and 24 hours prior to any study assessment visit; • Only for cohorts undergoing lumbar puncture (Cohorts 5 **to 8**): Artificial nails or nail polish on days of pulse oximetry during lumbar puncture (Days 2, 8, and 15). For safety reasons, subjects will be required to remain supine and avoid sleeping from approximately 1 hour before the start of the infusion until after the first 4 hours after start of study treatment infusion.
Blood Sample Collection for PK Analysis:	A total of 21 blood samples will be collected in each cohort for PK analysis: pre-start of infusion and 0.25 (±3 min), 0.5 (±3 min), 0.75 (±3 min), 1 (±3 min), 1.25 (±3 min), 1.5 (±3 min), 2 (±3 min), 4 (±3 min), 6 (±3 min), 8 (±3 min), 12 (±15 min), 24 (±15 min) (Day 2), 36 (±15 min) (Day 2), 48 (±15min) (Day 3), 96 (Day 5±1), 168 (Day 8±1), 336 (Day 15±1), 504 (Day 22±2), 672 (Day 29±2), and 1416 (Day 60±3) hours post-start of infusion.
Blood Sample Collection for PD Analysis:	A total of 14 blood samples will be collected in each cohort for PD analysis: pre-infusion and 1, 4, 6, 12, 24 (Day 2), 36 (Day 2), 48 (Day 3), 96 (Day 5±1), 168 (Day 8±1), 336 (Day 15±1), 504 (Day 22±2), 672 (Day 29±2), and 1416 (Day 60±3) hours post-start of infusion.
Blood Samples for ADA Assessment:	A total of 6 immunogenicity blood samples will be collected for ADA and NAbs: pre-start of infusion and at 96 (Day 5±1), 336 (Day 15±1), 504 (Day 22±2), 672 (Day 29±2), and 1416 (Day 60±3) hours post-start of infusion.

CSF Sample Collection:	Cohorts 5 **to 8** only: For each subject, one single CSF sample will be collected via lumbar puncture over the study, for PK and PD analysis. Subjects in each cohort will be randomized to have their CSF sample collected at one of the following timepoints, so as to have 2 subjects assigned to each timepoint: 24 (±3 hours; Day 2), 36 (±3 hours; Day 2), 168 (Day 8±1), or 336 (Day 15±1) hours post-start of infusion, according to the randomization scheme. Sentinel subjects will be randomized to the same timepoint. Placebo subjects will serve to evaluate change from baseline. For cohorts undergoing lumbar puncture (Cohorts 5 **to 8**), vital signs will be measured before lumbar puncture and will be measured approximately 0.5 hours after lumbar puncture, meaning approximately 24.5 (±3 hours; Day 2), 36.5 (±3 hours; Day 2), 168.5 (Day 8±1), or 336.5 (Day 15±1) hours post-start of infusion. Pulse oximetry (PO) will be performed during lumbar puncture: 24 (±3 hours; Day 2), 36 (±3 hours; Day 2), 168 (Day 8±1), or 336 (Day 15±1) hours post-start of infusion, according to the randomization scheme. A catheter will be inserted during the procedure for safety. Cumulative safety/tolerability data from early cohorts will inform on whether lumbar puncture will be started at Cohort 5.
Subject Monitoring:	Body weight: Day -1. Brief physical examination: Day -1, Day 3 (approximately 48 hours post-start of infusion), Day 8±1, Day 15±1, and as symptoms directed. BP, HR, RR, and OT: Day -1. BP, HR, and OT: Day 1 pre-infusion and 1.5, 2, 4, 6, 8, 12, 24 (Day 2), and 48 (Day 3) hours post-start of infusion; Days 5±1, 8±1, 15±1, 22±2, and 29±2. ECG: Day 1 pre-infusion and 1.5, 4, 8, 12, 24 (Day 2), and 48 (Day 3) hours post-start of infusion; Days 5±1, 8±1, 15±1, 22±2, and 29±2. Hematology, biochemistry, coagulation, and urinalysis: Day -1, Day 3 (approximately 48 hours post-start of infusion), and Days 8±1, 15±1, and 29±2. Alcohol breath test and urine drug screen: Day -1. Injection site evaluation: pre-infusion and approximately 0.5, 1, 24, 48, 96 (Day 5±1), and 168 (Day 8±1) hours post-start of infusion. When timepoints for ECGs, vital signs, and blood draws coincide, procedures will be carried out in the said order. Medical surveillance: Subjects will be monitored throughout the study by the clinical staff for AEs. In each cohort, the Investigator or designee will be on site one hour prior to the start of the treatment infusion and until 5 hours post-start of infusion, and available on call for the remainder of the study.
Study Exit Procedures or ET:	On the last study visit (Day 60 ±3): complete physical examination, hematology, biochemistry, coagulation, urinalysis, vital signs, ECG, S-STS, and AEs monitoring.
Analytical Methods:	NS101 in serum and in CSF, and samples of FAM19A5 in CSF and in plasma will be analyzed using validated methods. Immunogenicity (ADAs status and NAbs) will be assessed using a validated method. NAbs will be analyzed only for subjects with ADA positive results.

Pharmacokinetics:	Serum PK: AUC_{0-24}, AUC_{0-t}, AUC_{0-inf}, Residual area, C_{max}, T_{max}, $T_{½\ el}$, K_{el}, Cl, and V_z for NS101. CSF PK: A listing of all NS101 concentration in CSF will be presented. The ratio of NS101 in serum to NS101 in CSF will be also listed.
Pharmacodynamics:	Plasma PD (variation of level of FAM19A5 in plasma: Δ FAM19A5): $AUEC_{0-t}$, E_{max}, TE_{max} for FAM19A5. CSF PD: A listing of all FAM19A5 concentrations in CSF will be presented.
Statistical Analyses:	A complete description of the statistical analyses to be performed on safety, tolerability, PK, PD, and immunogenicity data will be presented in a Statistical Analysis Plan (SAP). Safety and Tolerability: Demographic parameters will be summarized descriptively. Safety and tolerability to NS101 will be evaluated through the assessment of AEs (i.e., seriousness, severity, relationship to the study drug, outcome, duration, and management), vital signs, 12-lead ECG, clinical laboratory parameters, weight, and physical examination. Treatment-emergent AE (TEAEs) will be tabulated by study treatment. Changes from baseline values in vital signs, ECG, and clinical laboratory parameters tabulated by study treatment will be evaluated. Safety and tolerability data will be reported using descriptive statistics. No inferential statistical analysis of safety data is planned. PK and PD: Individual and mean serum or plasma concentrations versus time curves will be presented for both linear and semi-log scales for NS101 and FAM19A5. Descriptive statistics for each dose level (arithmetic and geometric means, standard deviation [SD], coefficient of variation [CV (%)], minimum [Min], maximum [Max], and median) of the serum or plasma concentrations versus time will be presented as well for the PK and PD parameters. Interim PK analyses will be performed for each dose level using the serum concentrations data. Summary statistics will be used to describe the PK profile for this cohort. Dose proportionality may be assessed within different dose ranges if deemed appropriate with at least three doses using the power model approach. Dose-response relationship may be performed using Logistic Regression plots to assess the relationship between PK data after single dose administrations and specific AEs, when data permitting. After database lock, the power model approach will also be performed on AUC_{0-24}, AUC_{0-t}, AUC_{0-inf}, and C_{max} data to assess the dose-proportionality when all dose levels will be completed. Non-parametric analysis will also be performed on the untransformed T_{max} data.

	Immunogenicity: Incidence and titer of ADAs and NAbs will be assessed.

4. Schedule of Events

PROCEDURE	Screening D-28 to D-2	Cohorts 1 to 8[1]									Study Exit (Day 60 ± 3) or E.T.
		D-1	D1	D2	D3	D5±1	D8±1	D15±1	D22±2	D29±2	
Informed Consent	X										
Demographic Data	X										
Medical and Medication Histories	X										
Review and Monitoring of AEs and Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X
Physical Examination[2]	X	X			X		X	X			X
Body Measurements[3]	X	X									
Vital Signs (BP, HR, RR, OT)[4]	X	X	X	X[13]	X	X	X[13]	X[13]	X	X	X
ECG	X		X[5]	X[5]	X[5]	X	X	X	X	X	X
S-STS	X										X
Hematology	X	X[6]			X		X	X		X	X
Biochemistry	X	X[6]			X		X	X		X	X
Coagulation	X	X[6]			X		X	X		X	X
Serology (HIV and Hepatitis B and C)	X										
Urinalysis	X	X[6]			X		X	X		X	X
Urine Drug Screen	X	X									
Alcohol Breath Test	X	X									
Confinement		X	X	X	X						
Randomization			X[7]								
Injection Site Evaluation			X[8]	X[8]	X[8]	X[8]	X[8]				
Study Treatment Administration			X[9]								
Blood PK Samples[10]			X	X	X	X	X	X	X	X	X
Blood PD Samples[11]			X	X	X	X	X	X	X	X	X
Lumbar Puncture for CSF Samples[12]				X			X	X			
Pulse oximetry (PO)[14]				X			X	X			
Blood Samples for ADA Assessment[15]			X			X		X	X	X	X

1 Dose escalation will not occur until safety and tolerability up to Day 8±1 and available PK data up to 48 hours post-start of infusion for at least 7 subjects are assessed in the preceding cohort, and the study drug is deemed safe by the SRC.

2 A complete physical examination will be performed at screening and study exit. A brief physical examination will be performed on Day -1, Day 3 (approximately 48 hours post-start of infusion), Day 8±1, and Day 15±1, and as symptoms directed.

3 Height, weight, and BMI will be collected at screening. Body weight will be collected on Day -1 to calculate the dose to be administered per subject.

4 BP, HR, RR, and OT: screening, Day -1, and study exit. BP, HR, and OT: Day 1 pre-start of infusion and 1.5, 2, 4, 6, 8, 12, 24 (Day 2), and 48 (Day 3) hours post-start of infusion, as well as on Days 5 ±1, 8±1, 15±1, 22±2, and 29±2.

5 ECG: Day 1 pre-infusion and 1.5, 4, 8, 12, 24 (Day 2), and 48 (Day 3) hours post-start of infusion.

6 Hematology, biochemistry, coagulation, and urinalysis: Day -1. Laboratory assessments can be done at check-in or in the morning of Day -1.

7 Randomization will be done for treatment, sentinel, and lumbar puncture timepoints.

8 Injection site evaluation: before dosing and approximately 0.5, 1, 24 (Day 2), 48 (Day 3), 96 (Day 5±1) and 168 (Day 8±1) hours post-start of infusion.

9 Subjects will receive a single infusion of NS101 or matching placebo under fasting conditions over a period of approximately 1 hour. A staggered dosing schedule will be used and will include 2 sentinel subjects (1 active and 1 placebo) dosed initially, and the remaining subjects dosed no sooner than 48 hours after the start of sentinel dosing.

10 Blood samples for PK assessment: pre-start of infusion and 0.25 (±3 min), 0.5 (±3 min), 0.75 (±3 min), 1 (±3 min), 1.25 (±3 min), 1.5 (±3 min), 2 (±3 min), 4 (±3 min), 6 (±3 min), 8 (±3 min), 12 (±15 min), 24 (±15 min) (Day 2), 36 (±15 min) (Day 2), 48 (±15min) (Day 3), 96 (Day 5±1), 168 (Day 8±1), 336 (Day 15±1), 504 (Day 22±2), 672 (Day 29±2), and 1416 (Day 60±3) hours post-start of infusion.

11 Blood samples for PD assessment: pre-start of infusion and 1, 4, 6, 12, 24 (Day 2), 36 (Day 2), 48 (Day 3), 96 (Day 5±1), 168 (Day 8±1), 336 (Day 15±1), 504 (Day 22±2), 672 (Day 29±2), and 1416 (Day 60±3) hours post-start of infusion.

12 For Cohorts 5 **to 8** only: Lumbar puncture for CSF samples: One single CSF sample via lumbar puncture for PK and PD analysis at 24 (±3 hours; Day 2), 36 (±3 hours; Day 2), 168 (Day 8±1), or 336 (Day 15±1) hours post-start of infusion, according to the randomization scheme.

13 For Cohorts 5 **to 8** only, vital signs will be measured before lumbar puncture and will be measured approximately 0.5 hours after lumbar puncture, meaning approximately 24.5 (±3 hours; Day 2), 36.5 (±3 hours; Day 2), 168.5 (Day 8±1), or 336.5 (Day 15±1) hours post-start of infusion, according to the randomization scheme.

14 For Cohorts 5 **to 8** only, PO will be performed during lumbar puncture, at 24 (±3 hours; Day 2), 36 (±3 hours; Day 2), 168 (Day 8±1), or 336 (Day 15±1) hours post-start of infusion, according to the randomization scheme.

15 Blood samples for assessment of immunogenicity (ADAs and NAbs): pre-start of infusion and 96 (Day 5±1), 336 (Days 15±1), 504 (Day 22±2), 672 (Day 29±2), and 1416 (Day 60±3) hours post-start of infusion. NAbs will be analyzed only for subjects with ADA positive results.

5. Introduction

5.1 Background Information

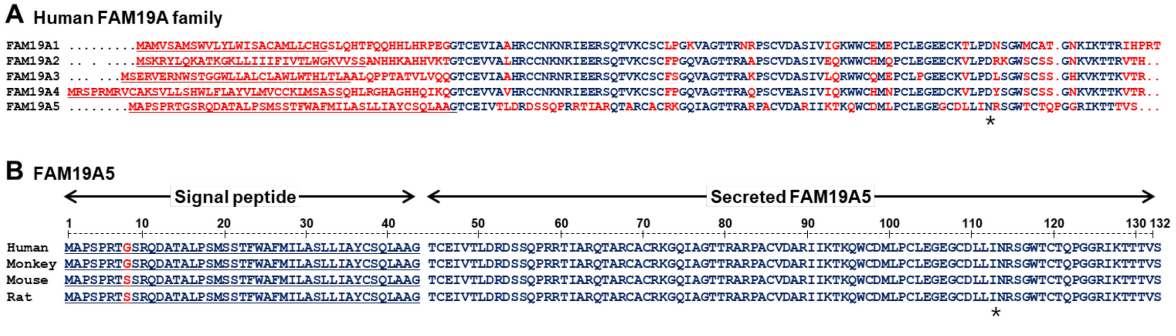
Neuracle Science is developing NS101 for the treatment of neurodegenerative diseases, including Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), and spinal cord injury (SCI).[1]

AD is a progressive neurodegenerative condition and the leading cause of dementia in older age. Existing treatment options consist of cognitive and behavioural interventions, acetylcholinesterase enzyme inhibitors (AChEIs), and N-methyl d-aspartate (NMDA) antagonists, provide symptomatic treatment only, but do not attenuate disease progression. ALS is a degenerative neurological disease affecting motor neurons in the brain and spinal cord. ALS treatment is predominantly symptomatic, with only two approved medications (riluzole, a glutamate antagonist, and edaravone, an antioxidant with neuroprotective effect) offering very modest disease modifying effects. SCI is a debilitating neurological condition. It has been recently demonstrated the importance of synaptic assembly in the restoration of neural circuits. Findings proposes that reconnecting synapses alone can promote restoring neuronal integrity which greatly helps in improving cognitive functions and motor skills.[1]

5.1.1 FAM19A5 Target

FAM19A5 (Family with sequence similarity 19, member A5) was identified as a transformative, therapeutic target involved in a suite of neurological disorders using a bioinformatic approach. FAM19A5 is found from most of mammals, such as humans, mice and rats. FAM19A5 proteins exhibit a high degree of amino acid (aa) sequence identity across vertebrate species, suggesting that they may have similar physiological and molecular behaviors in terms of FAM19A5-mediated pathways (Figure 1).[1]

Figure 1. Amino Acid Sequence Alignment of the FAM19A Family



(A) Amino acid (aa) sequence alignment of the human FAM19A family. Signal peptide sequences are underlined. The conserved aa residues across 5 members are in blue whereas variable residues are in red. (B) Amino acid sequence alignment of mammalian FAM19A5. Human, monkey, mouse, and rat FAM19A5 aa sequences are retrieved from Ensemble Genome Browser (http://asia.ensembl.org). The full length of FAM19A5 consists of signal peptide sequence followed by matured secreted FAM19A5. Note that only one aa difference at position 8 is observed among the sequences as indicated in red. *, putative residue for N-glycosylation.

FAM19A5 plays an inhibitory role in synaptic assembly, contributing to fine-tuning between synaptic assembly and disassembly. FAM19A5 binds tightly with key postsynaptic neuronal organizer leucine-rich repeat-containing protein 4B (LRRC4B). Binding between FAM19A5 and LRRC4B is highly associated with dynamic loss of synapses, downregulating synaptic function.[1]

FAM19A5 proteins are abundantly expressed in the central nervous system (CNS), including various brain tissues, spinal cord, and dorsal root ganglia (DRG) in the peripheral nervous system. They are expressed at much lower levels in peripheral tissues, including in female and male reproductive tissues, endocrine tissues, and some cardiac and smooth muscle cells. FAM19A5 protein synthesized in the brain can be secreted into the CSF, and is present in significant quantities in rodent and human CSF. FAM19A5 levels in CSF display an age-dependent increase, and ELISA has demonstrated a positive correlation between FAM19A5 and typical neurodegenerative disease biomarker levels, including t-/p-tau, YKL-40, and S100β proteins.[1]

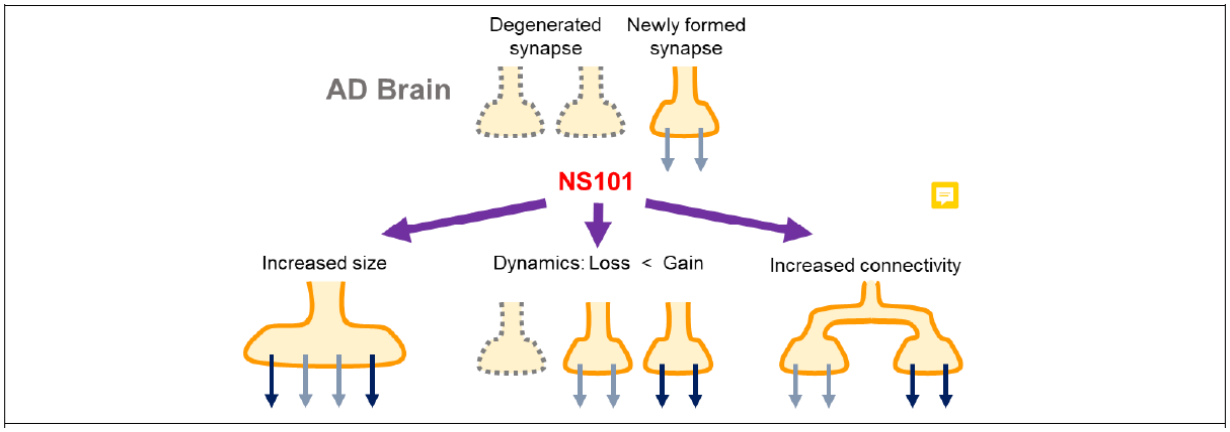
5.1.2 NS101 Lead Candidate

NS101 is the lead candidate in a series of chimeric chicken/human monoclonal antibodies (mAb) to neutralize FAM19A5, being developed by Neuracle Science. The product is produced in human immunoglobulin G1 (hIgG1) generated through the immunization of chickens (*gallus gallus domseticus*) with purified recombinant FAM19A5. NS101 is an IgG1 antibody with a molecular weight of approximately 146 kDa. Each light chain consists of 214 amino acid residues and each heavy chain is composed of 461 amino acid residues. NS101 specifically binds to FAM19A5 with affinity (K_d values) of ~100 pM. NS101 was optimized to recognize a specific epitope at the N-terminus of FAM19A5. Mutation in a region results in loss of binding to receptors (FcγRs) of the immune cells and consequently, no or limited FcγR-mediated effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC), without having any impact on the affinity of NS101to neonatal Fc receptor (FcRn).[1]

As circulating NS101 reach FAM19A5 in the brain parenchyma after traveling across the blood brain barrier, NS101 forming a complex with FAM19A5 can be wired into the CSF, affecting FAM19A5 concentrations in the CSF. The clearance rate of CSF FAM19A5 can be affected by the convective flow that secretes NS101 back into the peripheral circulation. The complex formation occurs immediately after IV administration of NS101, peaks at 24 hours after administration, and time-dependently decreases, indicating a correlation between the complex formation and left-over NS101 concentration.[1]

In preclinical data, NS101 has been found to promote synapse formation of primary neurons both *in vitro*, and *in vivo* in the hippocampus in mouse models of AD, via mediating dissociation of the FAM19A5+LRRC4B complex. In the AD brain, increased loss of synapses overwhelms gain of synapses, causing a net reduction in synaptic capacity. NS101 may increase synaptic capacity through increasing size of remaining synapses, altering synapse dynamics to increase synapse gain over synapse loss, and/or enlarging connections within existing neuronal networks. NS101-induced increases in synaptic transmission are indicated by cobalt blue arrows below (Figure 2).[1] In ALS mice, NS101 showed protection against volume loss in the brain and lumbar spinal cord followed by substantial improvements in motor function. Three main observations have been noted and are presented in Figure 2.[1]

Figure 2. Proposed Hypothesis Explaining How NS101 Increases Synaptic Capacity



5.2 Summary of Nonclinical Data

5.2.1 Nonclinical Pharmacology

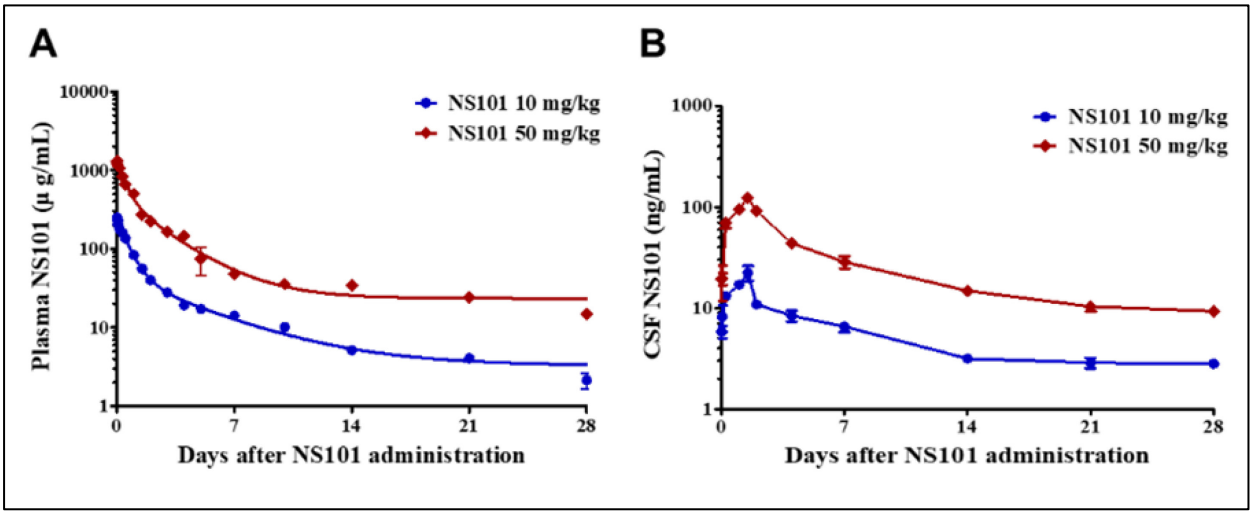
5.2.1.1 In-vitro Studies

In-vitro studies demonstrated that NS101 can effectively dissociate the FAM19A5+LRRC4B complex. Details are presented in the Investigator's Brochure.[1]

5.2.1.2 In-vivo Studies

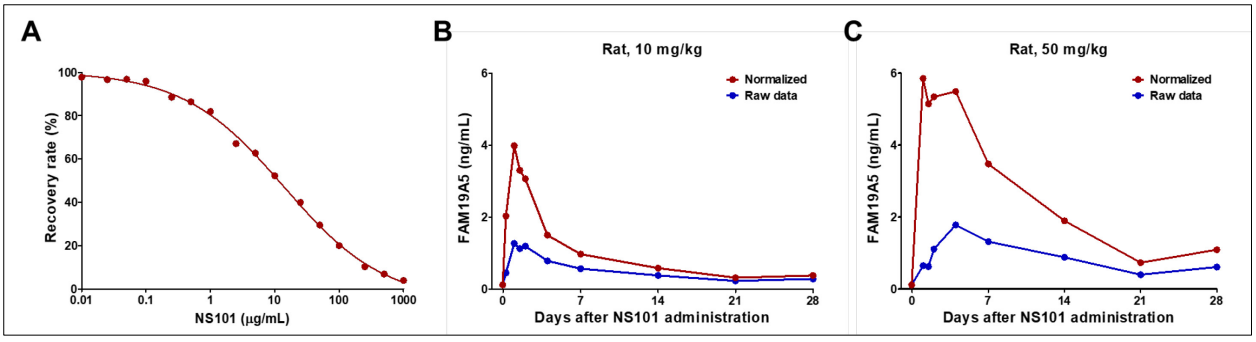
A key hypothesis is that NS101 can engage target FAM19A5, hijack, and migrate it into the blood stream by the complexation, altering FAM19A5 levels in the CSF and blood. To characterize FAM19A5 levels in each compartment, time-course changes in NS101 levels were determined in the CSF of male rats IV administered with 10 or 50 mg/kg NS101 using ELISA. NS101 levels increased immediately after administration. Substantial levels of NS101 were detected 5 minutes after NS101 administration in both 10 and 50 mg/kg treated groups. CSF NS101 levels increased rapidly up to 8 hours. NS101 levels then rose slowly and peaked at 36 hours after administration. During the log and stationary phases, NS101 levels maintained an approximately 5-fold difference between 10 and 50 mg/kg-treated groups. After peaking at 36 hours post-dose, CSF NS101 levels continued to decrease, which may reflect the decrease in NS101 (Figure 3).[1]

Figure 3. NS101 Concentrations in Plasma and CSF Following IV Administration into Rats



The recovery rate of FAM19A5 in the presence of escalating concentrations of NS101 (0.01 ~ 1,000 μg/ml) was investigated. NS101 concentrations within the ranges are detected in the blood when NS101 was IV administered. IV administration of NS101 to naïve rats had dramatic increases in blood FAM19A5 levels within 6 hours in a dose- and a time-dependent manners. For rats treated with 10 mg/kg NS101, FAM19A5 levels in the blood reaches its peak at 36 ~ 48 hours after NS101 administration and then gradual decreases. For rats treated with 50 mg/kg NS101, the increase in blood FAM19A5 level was delayed compared to that in rats treated with 10 mg/kg NS101, and then the level declined based on raw data. Normalized levels of FAM19A5 peaked at 24 hours and stayed in the stationary phase until 4 days after 50 mg/kg NS101 administration (Figure 4). The result points to the notion that the FAM19A5 maximum level in the blood of rats might be 5.5~6 ng/mL given that the movement of FAM19A5 from the brain to the blood stream caused by NS101 is saturated.[1]

Figure 4. NS101-Induced Blood FAM19A5 Levels in Rats

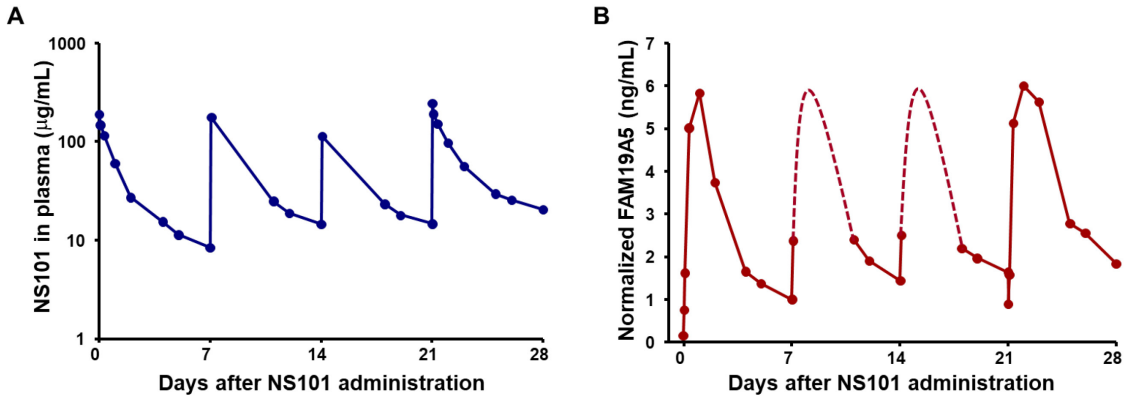


(**A**) Recovery rates of FAM19A5 in the presence of NS101. FAM19A5 levels were measured using rat plasma spiked with 5 ng/mL FAM19A5 under increasing concentrations of NS101 (0.01, 0.025, 0.5, 1, 2.5, 5, 10, 25, 50, 100, 250, 500, and 1,000 μg/mL). Recovery rate was displayed compared to FAM19A5 levels in the absence of NS101. A range of NS101 doses showing linear inhibition was selected to plot a standard inhibition curve that is used to normalize FAM19A5 levels under various concentrations of NS101 in the blood. (**B, C**) Plasma FAM19A5 levels in rats IV administered with NS101 of **(B)** 10 mg/kg or **(C)** 50 mg/kg. Plasma samples were collected at indicated time points for 28 days. Plasma FAM19A5 concentrations (raw data) were measured using a FAM19A5-detecting ELISA. FAM19A5 levels (raw data) were recalculated using the standard inhibition curve by applying

NS101 concentration detected in plasma samples and the data were expressed as normalized FAM19A5. All data are expressed as the mean of three rat samples.

The blood NS101 and FAM19A5 levels were investigated after repeated, weekly administration of 10 mg/kg NS101 into rats. NS101 levels in the blood were time-dependently reduced after each dosing. For normalized FAM19A5 levels in the blood, the level reached a peak on Day 1.5 followed by gradual decrease until Day 7 after each regimen. The results indicate that blood FAM19A5 can be a PD marker to predict NS101 action (Figure 5).[1]

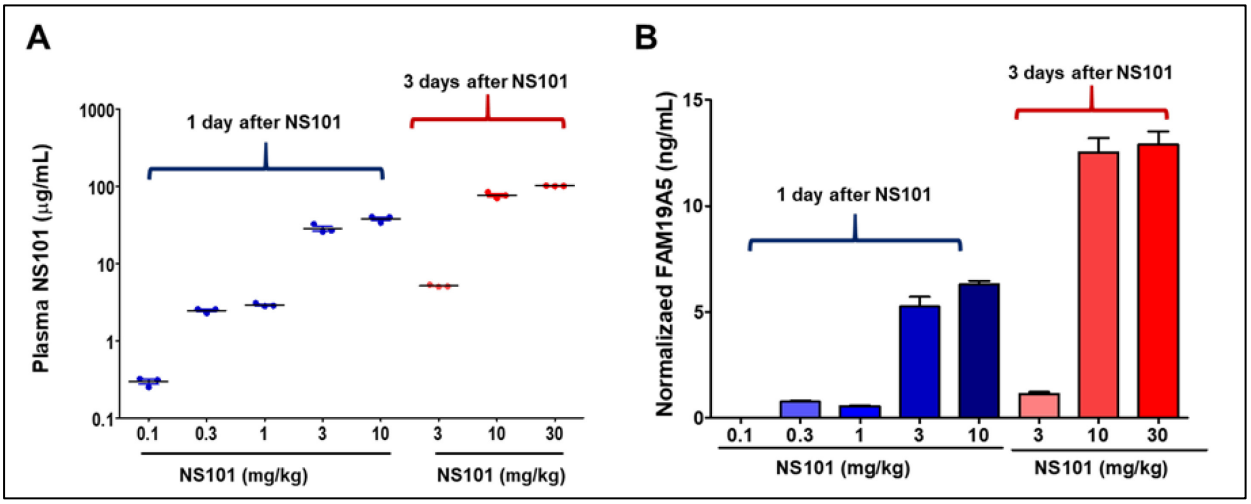
Figure 5. NS101 and FAM19A5 Levels in Blood after Repeat IV Dosing in Rats



Rats were IV dosed weekly for 4 weeks with NS101 of 10 mg/kg. Plasma was collected at indicated time points for 28 days. (**A**) Plasma NS101 concentrations were measured using ELISA with rabbit anti-human IgG Fc and HRP-conjugated goat anti-human IgG kappa light chain antibodies. (**B**) Plasma concentrations of FAM19A5 were measured by a Sandwich ELISA with an LRRC4B fragment and 1-65 antibody. The raw data were recalculated to obtain normalized levels of FAM19A5 using the standard inhibition curve. Data are expressed as the mean value of data from 7 rats.

The NS101 concentration that is minimally required for FAM19A5 release into peripheral blood and the concentration that elicits the saturated circulation of FAM19A5 in peripheral compartments was further investigated. Mice were IV dosed with NS101 from a dose of 0.1 mg/kg to 30 mg/kg. The increase in blood FAM19A5 levels was initially identified in mice treated with 0.3 mg/kg NS101 and FAM19A5 levels were elevated depending on the NS101 dose at 1 day after NS101 treatment (Figure 6). For mice treated with 3 mg/kg NS101 exhibited the significant reduction in FAM19A5 levels 3 days after NS101 treatment compared to the FAM19A5 levels 1 days after treatment. However, mice treated with 10 mg/kg NS101 showed higher FAM19A5 levels 3 days after treatment than 1 day after treatment. FAM19A5 levels 3 days post-treatment with NS101 were similar between the dose of 10 and 30 mg/kg NS101.[1]

Figure 6. NS101-Induced Blood FAM19A5 Levels In Mice



Blood FAM19A5 levels in mice iv administered with NS101 (SE1901) of 0.1, 0.3, 1, 3, 10, or 30 mg/kg. Plasma samples were collected at day 1 (for 0.1, 0.3, 1, 3, and 10 mg/kg NS101-treated group, **left in blue**) and day 4 post-administration with NS101 (for 3, 10, and 30 mg/kg NS101-dosed group, **right in red**). **(A)** Plasma NS101 and **(B)** FAM19A5 concentrations were measured using ELISA. Data are expressed as the mean of SEM of three mouse samples.

The NS101 dose that minimally induces FAM19A5 release is 0.3 mg/kg, which is 1/33 of the 10 mg/kg dose. This observation allows a simple calculation to estimate the minimum plasma NS101 concentration required for FAM19A5 release. For instance, plasma NS101 concentrations immediately after IV administration of 10 mg/kg NS101 range from 200 to 300 μg/mL in rats and monkeys, so 1/33 of this concentration is 6 to 9 μg/mL, which are likely the minimum concentration of NS101 to drive the FAM19A5 release.[1]

5.2.2 Safety Pharmacology

One study has been conducted in 32 male Sprague-Dawley rats to evaluate the potential modifications of CNS activity by NS101 after a single IV administration to conscious rats. No significant changes were recorded in neurologic, autonomic or behavioral responses at 50, 150, or 300 mg/kg.[1]

The potential effect of the test item, NS101, on cardiovascular and respiratory functions was evaluated after single intravenous infusion (IV, 1 hour in a restraint chair) at two dose levels to conscious cynomolgus monkeys. No mortality occurred during the study. No test item-related changes were observed in arterial blood pressure, PQ interval, QRS interval, QTca interval, lead II ECG waveform morphology, and respiratory parameters. Administration of NS101 at 300 mg/kg was associated with decreases in heart rate (with concomitant increases in QT interval) up to 22 hours with a maximum decrease of 83.7 bpm at 9 hours post-dose. Decreases in body temperature were present up to 18 hours following administration of NS101 at 300 mg/kg, with a maximum decrease of 0.8°C between 9 and 10 hours post-dose. IV infusion of NS101 at 90 mg/kg had no effect on cardiovascular function, cardiac electrophysiology or respiratory function in conscious cynomolgus monkeys. The no observed effect level (NOEL) for cardiovascular and respiratory effects was considered to be 90 mg/kg NS101, based on the effects noticed on heart rate and body temperature at 300 mg/kg NS101.[1]

5.2.3 Nonclinical Toxicology

5.2.3.1 A 14-Day Repeated IV Dose Range Finding Toxicity and Toxicokinetic Study of NS101 in Sprague-Dawley Rats

For toxicity group, a total of 40 male and female Sprague-Dawley rats were allocated to four groups received the vehicle or the test item, NS101, by IV short infusion at the dose levels of 50, 150, and 400 mg/kg into the tail vein at dose volume of 20 mL/kg, weekly for 14 days.[1]

There were no deaths or treatment-related abnormalities in clinical signs, body weights, food consumption, hematology, clinical chemistry, organ weights, and gross post-mortem examination.[1]

Histopathological examination revealed that in the spleen, mild lymphoid hyperplasia in white pulps and/or marginal zones was noted in the treatment groups of both male and female rats, which was comparable to no incidence in the vehicle control groups of both genders. Thus, the lesion was likely to be related to the treatment of test material, although the severity was usually minimal to mild. The toxicological significance of the lesion was not clear in the study, but it might be more associated with inflammatory reaction in any other organ rather than the direct effect to the spleen when consider that white blood cell (WBC) count was significantly increased in the treatment groups.[1]

In the large intestine, multifocal increase of eosinophil infiltration in the lamina propria of mucosa and submucosa was found in some of the male and female treatment groups without evidence of other lesions. Based on the incidence of the lesion, the possibility related to the treatment of test material was not excluded, but it might not be so significant in toxicology because there was no evidence of other histological features of inflammation. It was also observed in the vehicle control group. Mild infiltration of eosinophils and other inflammatory cells such as lymphocytes and plasma cells were common in the lamina propria of mucosa and submucosa in rat gastrointestinal tracts.[1] No treatment-related specific lesions were observed in the bone marrow of treatment groups of male and female rats as well as in the vehicle control group.[1]

The toxicokinetics results following IV administrations of NS101 at 50, 150 and 400 mg/kg to male and female rats, the systemic exposure, in terms of C0 and AUClast, increased over the 50 to 400 mg/kg/dose dosing range. The increase in exposure was a dose-proportional manner on Days 1 and 15 (Table 1 and Table 2). Accumulation was not observed following 14 days of intravenous administration.[1]

Table 1. Toxicokinetic Parameters of NS101 following Intravenous Administration to Sprague-Dawley Rats on Day 8

Group/ Treatment	Dose Level (mg/kg/dose)	Gender	C0 µg/mL	t1/2 hr	AUClast ug*hr/mL	AUC0-168 ug*hr/mL	AUCinf ug*hr/mL	%AUCextra %	CL mL/hr/kg	Vdss mL/kg
G2 NS101	50	Male	1039.9±47.6	53.7±1.3	70100.9±2113	NA	79547.3±1777.1	11.9±0.7	0.6±0	37.4±2.3
		Female	959.6±35.8	61.3±2.4	69461±3245.1	NA	82546.1±4832.1	15.8±1.1	0.6±0	44.8±1.5
G3 NS101	150	Male	2859.5±260.1	61.3±5.2	213317.9±8504.5	NA	251614.9±11109.7	15.2±2	0.6±0	44±5.3
		Female	2677.6±143.6	67.5±2.1	208001.5±9730.1	NA	255319.9±8970.8	18.6±1.2	0.6±0	50.2±3.8
G4 NS101	400	Male	7571.1±115.2	56.5±1.1	537827.2±10680.7	NA	618065.6±12491.6	13±0.6	0.6±0	42.3±1.2
		Female	7915.6±216.9	60.6±6.4	577089.9±20472.8	NA	681438.9±49571.3	15.2±3.2	0.6±0	42.5±3.8

NA: Not applicable

Table 2. Toxicokinetic Parameters of NS101 following Intravenous Administration to Sprague-Dawley Rats on Day 15

Group/ Treatment	Dose Level (mg/kg/dose)	Gender	C0 µg/mL	t1/2 hr	AUClast ug*hr/mL	AUC0-168 ug*hr/mL	AUCinf ug*hr/mL	%AUCextra %	CL mL/hr/kg	Vdss mL/kg
G2 NS101	50	Male	1243.5±80.6	281.5±23.0	110254.3±4109.7	59213.7±1615.6	130228.5±3917.0	15.3±1.3	0.4±0.0	126.9±7.2
		Female	1096.2±72.4	218.9±34.5	104981.9±6300.3	54559.8±4073.2	117742.8±9182.9	10.7±3.5	0.4±0.0	119.4±14.0
G3 NS101	150	Male	3516.3±282.1	222.0±16.4	313780.4±22621.8	175811.5±7375.6	348621.2±30093.4	9.9±1.3	0.4±0.0	113.5±1.3
		Female	3149.5±155.5	178.7±21.8	299436.0±15747.8	167836.8±2830.4	321841.8±24670.9	6.8±2.5	0.5±0.0	106.5±7.6
G4 NS101	400	Male	8092.8±673.6	197.4±11.3	674202.2±11893.3	407321.0±8933.6	727095.0±19196.2	7.3±0.9	0.6±0.0	123.5±1.6
		Female	7668.8±125.6	169.8±6.2	691195.4±67622.3	407511.1±41342.0	731555.5±74627.7	5.5±0.5	0.6±0.1	115.1±9.5

Sprague Dawley rats at dose levels 50, 150 and 400 mg/kg/dose for 14 days resulted in no adverse effects, therefore the maximum tolerated dose (MTD) of NS101 was considered to be > 400 mg/kg/dose for males and females.[1]

5.2.3.2 4-Week Toxicity Study by IV Administration in Rats Followed by a 4-Week Treatment-Free Period

Four groups of Sprague-Dawley rats, each comprising 18, 16 or 21 animals per sex, were administered the test item, NS101 at 0, 50, 150 or 300 mg/kg/dose in buffer, by once weekly IV injection for 4 weeks. Five males and five females in the control and high-dose groups were kept for an additional 4-week treatment-free period (recovery animals). Satellite animals (three or six rats/sex/group) were included for toxicokinetic evaluation. NS101 was quantifiable over the sampling interval (15 minutes to 168 hours post-dose) at all doses and on both Day 1 and Day 22 occasions (Table 3).[1]

Table 3. Mean Toxicokinetic Parameters of NS101 Following 5 Minute-Intravenous Infusion of NS101 at 50, 150, or 300 mg/kg in Male and Female Sprague-Dawley Rats for 4 Weeks

Dose level (mg/kg)	Sex	Period	C(0) (ng/mL)	t_{last} (h)	AUC_{0-t} (h*ng/mL)	AUC_{0-t}/Dose ((h*ng/mL)/(mg/kg))	SE AUC_{0-t} (h*ng/mL)	Rac	M/F
50	Female	Day 1	997000	168	28300000	567000	1530000	_	_
50	Female	Day 22	1280000	168	74500000	1490000	1630000	2.63	_
50*	Male	Day 1	652000	168	26400000	527000	1950000	_	0.930
50	Male	Day 22	1210000	168	70300000	1410000	4220000	2.67	0.944
150	Female	Day 1	2780000	168	110000000	733000	3110000	_	_
150*	Female	Day 22	3530000	168	232000000	1550000	9690000	2.11	_
150	Male	Day 1	3010000	168	105000000	697000	1230000	_	0.951
150	Male	Day 22	4450000	168	243000000	1620000	14900000	2.32	1.05
300	Female	Day 1	5820000	168	249000000	832000	10500000	_	_
300	Female	Day 22	8430000	168	436000000	1450000	36400000	1.75	_
300	Male	Day 1	5720000	168	217000000	724000	5590000	_	0.871
300*	Male	Day 22	4930000	168	414000000	1380000	26100000	1.90	0.949

*: C(0) could not be determined by log-linear regression of the first two measurements, and was set to the first observed measurement; M/F: Male-to-Female ratio; Rac: Accumulation ratio; _: not analysed.

An increase in systemic exposure to NS101 was observed on Day 22 in all groups, indicating accumulation. Mean accumulation ratios ranged from 1.75 to 2.67. No marked difference was observed between male and female rats. The M/F AUC ratios ranged from 0.871 to 1.05. A slightly more than a dose-proportional increase was observed in the range of administered doses on Day 1 while a relative good dose proportionality was observed at Day 22.[1]

In terms of immunogenicity, almost all samples were found to be negative to ADA during the screening assay, with the exception of 5/219 samples (2.3%) for which positivity was detected, with signals very close to the cut-point. Three of them belong to the control group and the remaining positive samples were collected at pre-test. Therefore, they were not considered related to the administration of test item.[1]

For all samples where the NS101 concentrations were > 1.25 µg/mL (drug tolerance threshold defined during the method validation) and that were determined to be immunogenicity negative, drug interference with the detection of anti-NS101 antibodies cannot be excluded.[1]

The clinical observation results revealed that:

- All animals survived until scheduled necropsies.
- There were no test item-related effects on clinical signs, food consumption, ophthalmology, urinalysis or macroscopic and microscopic examinations.
- Body weight gain was minimally to slightly higher over the treatment period in males and females at 150 and 300 mg/kg/dose.
- Clinical pathology findings at the end of the treatment period consisted in higher lymphocyte counts (up to 2-fold) in females at ≥ 50 mg/kg/dose and males at 300 mg/kg/dose; higher reticulocyte counts in females at 300 mg/kg/dose (+42%); shortened activated partial thromboplastin and prothrombin times (up to -19%) in females

at ≥ 50 mg/kg/dose and males at ≥ 150 mg/kg/dose; decreased albumin to globulin ratio (up to -12%); lower cholesterol levels in females at ≥ 50 mg/kg/dose (up to -27%) and higher triglyceride concentrations in females at ≥ 150 mg/kg/dose (up to +83%).

- Organ weight changes included a decrease in the heart weight in males at 300 mg/kg, and an increase in the liver weight in males and females at ≥ 150 mg/kg/dose, without histopathological correlations. No other test article-related organ weight changes were noted. There were also no other indications of stress, which could otherwise possibly explain increased weight of the adrenal gland.

- Based on the clinical dosing planned in the upcoming first in human trial as a single dose, the repeated dose observation will not be clinically significance.[1]

None of these changes were considered to be adverse and they were all reversible at the end of the 4-week treatment-free period, except for liver weights in males that were still increased compared to controls, but there was no statistically significant change in absolute liver weight at recovery.[1]

There were other statistically significant changes in heart weight and thymus weights at recovery, but these changes were considered incidental. There was a statistically significant lower heart weight relative to body weight in the 300 mg/kg/dose females. Since this change in relative heart weight did not affect the absolute weight, had no microscopic correlation; was within historical control range, and was not present at the principal study, this isolated change was not considered test article-related. There were increased thymus weights (absolute and relative to body weight) in 300 mg/kg/dose males. Since this change was within historical control range, had no microscopic correlation, and was in a direction opposite to that normally associated with a toxic effect, the higher thymus weights were considered unrelated to test article administration.[1]

Administration of NS101 by once weekly IV injections for 4 weeks to Sprague-Dawley rats at 50, 150 or 300 mg/kg/dose resulted in no adverse findings. Consequently, under the experimental conditions of the study, the no observed adverse effect level (NOAEL) was set at 300 mg/kg.[1]

5.2.3.3 4-Week Toxicity Study by Intravenous Infusion in Cynomolgus Monkeys followed by A 4-Week Treatment-Free Period

Four groups of cynomolgus monkeys were administered weekly by a 1-hour intravenous infusion at 0, 30, 90 or 300 mg/kg of NS101. At the end of the dosing period, three animals/sex/group were necropsied. Two animals/group/sex at 0 (vehicle) and 300 mg/kg/adm were retained undosed for 4 weeks and then necropsied.[1]

No unscheduled deaths occurred during the study. No test item-related systemic clinical signs or local reactions were observed during the study. Minimal and reversible body weight loss was observed in NS101-administered animals at ≥ 90 mg/kg/adm. Other effects are described in the Investigator's Brochure.[1]

Under the experimental conditions of the study, the NOAEL was set at 300 mg/kg/adm, corresponding to a mean systemic exposure (AUC_{0-t}) of 138.5 mg.h/mL.[1]

NS101 of 300 mg/kg/dose at week 8 displayed neutralizing ADA in all tested cynomolgus monkeys. Nevertheless, this led to no immunologically significant findings because the microscopic assessment showed no observed immunogenicity and/or immune complex deposition during the recovery period (Table 4).[1]

Table 4. Summary Of ADA Response And Consequence

Dose level (mg/kg)	Week 4 (At the end of final treatment)				Week 8 (4 weeks after the last injection)			
	Tested positive	Significant titer elevation	Positive neutralizing effect	n	Tested positive	Significant titer elevation	Positive neutralizing effect	n
0	1	ND	ND	10	1	ND	ND	4
30	2	ND	ND	6	NA	NA	NA	0
90	1	1	1	6	NA	NA	NA	0
300	1	1	1	10	4	4	4	4

The toxicokinetic results are described in the Investigator's Brochure.[1]

Due to the important presence of ADAs, particularly NAbs, the PK and safety data from this study were not used for study design of the upcoming FIH study.

5.2.4 Pharmacokinetic Data in Animals

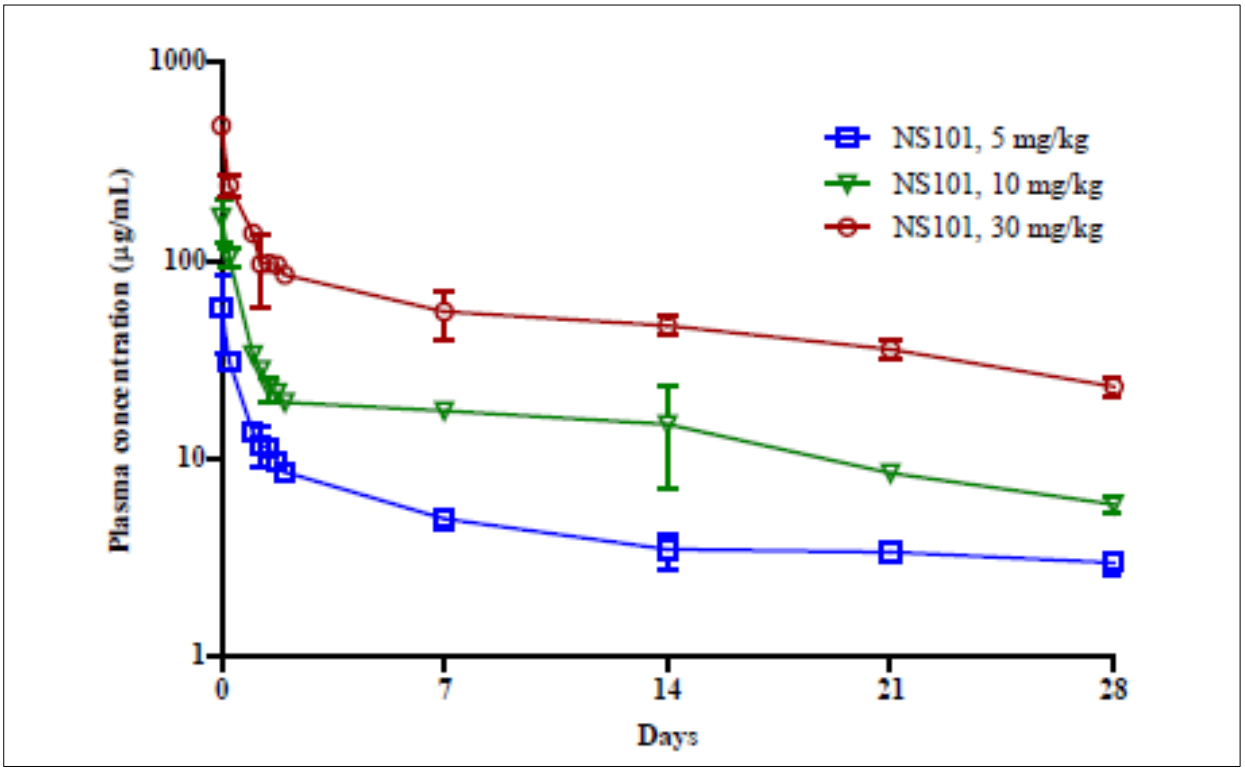
5.2.4.1 A PK Study of NS101 Following Single IV Administration to C57BL/6 Mice

NS101 was administered to mice at 5, 10 and 30 mg/kg via IV route. Plasma concentration of NS101 decreased with a mean terminal half-life ($T_{1/2}$) ranging from 249.6 to 329.9 hours. NS101 was uniformly distributed in circulating blood and tissue. Overall, AUC_{last} for NS101 was increased in a greater than dose proportional manner. Specifically, as the dose increased in a ratio of 1 : 2 : 6, the AUC_{last} increased in a ratio of 1 : 3 : 10.3, respectively (Table 5 and Figure 7).[1]

Table 5. The Mean Plasma PK Parameters of NS101 Following IV Administration of C57BL/6 Mice

PK parameters	NS101		
	5 mg/kg	10 mg/kg	30 mg/kg
C0 (µg/mL)	59.4	166.1	484.5
AUClast (µg·hr/mL)	3585.3	10750.7	36866.73
AUCinf (µg·hr/mL)	4990.2	12875.3	47908.2
t1/2 (hr)	324.6	249.6	329.9
CL (mL/hr/kg)	1.0	0.8	0.6
Vdss (mL/kg)	485.3	277.9	279.4
%AUCextra (%)	0.8	1.0	1.0
Rsq_adjusted	28.2	16.5	23.1

Figure 7. Mean (± SD) Plasma Concentrations Parameters of NS101 Following Single IV Administration to C57BL/6 Mice

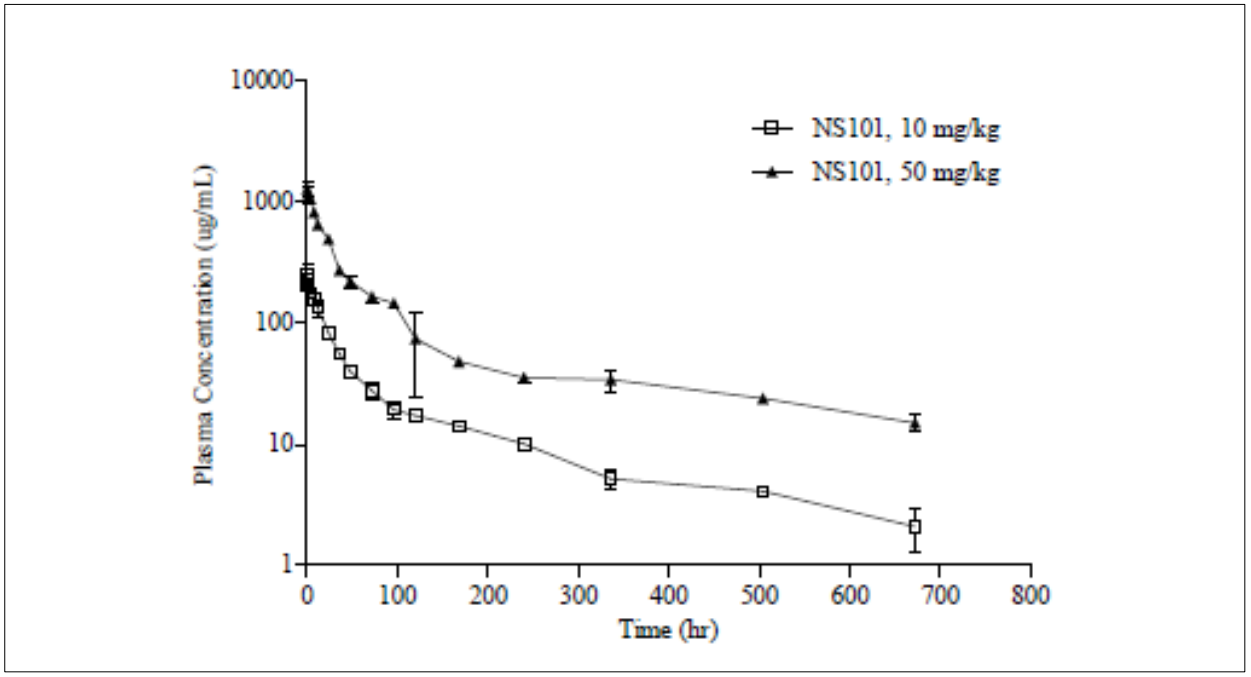


C57BL/6 mice were IV dosed once weekly for 4 weeks with NS101 of 5, 10 and 30 mg/kg. Plasma was collected at indicated time points for 28 days. Plasma NS101 concentrations were measured using ELISA with rabbit anti-human IgG Fc and HRP-conjugated goat anti-human IgG kappa light chain antibodies.

5.2.4.2 A Pharmacokinetic Study of NS101 Following Single IV Administration to Sprague-Dawley Rats

NS101 was administered to rats at 10 mg/kg and 50 mg/kg via IV route. Plasma concentration of NS101 decreased with a mean terminal half-life ($T_{1/2}$) ranging from 181.0 to 281.6 hours. NS101 was more distributed into circulation blood than tissues. The PK of NS101 was linear in the test dose range. (Figure 8).[1]

Figure 8. The Mean Plasma Concentrations-Time Profiles of NS101 Following Single IV Administration to Sprague-Dawley Rats



5.2.4.3 A PK Study of NS101 Following Repeated Intravenous Administration to Sprague-Dawley Rats

On Weeks 1 and 12, the plasma concentration of NS101 slowly decreased in a biphasic manner with the mean terminal half-life ($T_{1/2}$) ranged from 78.0 to 83.5 hours. NS101 was more distributed into circulation blood than tissues. Minimum concentration (C_{min}) slightly increased and reached steady-state on Week 4. Accumulation after repeated dose was observed and accumulation ratio (AUC_{last} ratio) was 2.0 (Table 6).[1]

Table 6. Summary of Pharmacokinetic Parameters of NS101 following Repeated IV Administration to Sprague-Dawley Rats

PK parameters	Pharmacokinetic Parameters											
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
AUClast (hr·μg/mL)	5418.2	11124.7	7917.5	12758.0	13293.4	14117.0	13978.3	14373.7	15032.3	15564.7	14726.0	10768.5
AUCinf (hr·μg/mL)	6369.1	12769.3	9834.0	14858.6	15466.1	15909.3	16508.5	16860.8	17730.2	18513.7	17453.1	13113.0
CL (mL/hr/kg)	1.6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.8
Vdss (mL/kg)	121.9	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	68.9
t1/2 (hr)	78.0	78.1	89.7	74.4	70.8	59.2	89.3	80.4	89.5	97.1	94.5	83.5
C0 (μg/mL)	196.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	313.3
Cmin (μg/mL)	8.5	14.6	14.7	20.0	21.3	20.6	20.7	21.9	20.7	21.0	19.8	19.7
%AUCexp (%)	15.0	12.7	18.7	14.1	13.8	11.1	15.2	14.5	15.0	16.0	15.6	17.7
Accumulation ratio[1)]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2.0

1): AUClast week12/ AUClast week1
NA: Not Applicable

5.2.4.4 4-Week Repeated Dose Toxicokinetics Study by IV Administration in Rats

A total of 42 rats (21/sex) were randomly assigned to 4 groups and were administrated with vehicle or NS101 at dosage of 50, 150 or 300 mg/kg/dose once weekly by IV infusion for 4 weeks (Table 7). There were no unscheduled death or treatment-related abnormalities in clinical signs and body weights.[1]

Table 7: Summary of PK Parameters Following Weekly IV Infusion of NS101

Dose (mg/kg/dose)	Study Day	Sex	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0-24.083h}$ (h*ng/mL)	$AUC_{0-168.083h}$ (h*ng/mL)
50	1	Male	1390000	0.3	19200000	49800000
		Female	1240000	0.3	17500000	46600000
	22	Male	1930000	2.1	31900000	102000000
		Female	2020000	0.3	27800000	93900000
150	1	Male	4430000	0.3	62500000	168000000
		Female	4600000	0.3	61600000	191000000
	22	Male	5770000	0.3	90000000	293000000
		Female	5550000	2.1	86000000	298000000
300	1	Male	8830000	0.3	132000000	383000000
		Female	8900000	0.3	120000000	393000000
	22	Male	11600000	0.3	190000000	617000000
		Female	11000000	0.3	166000000	570000000

After single (Day 1) or repeated (Day 22) IV infusion of NS101 to male and female rats, T_{max} values for NS101 were observed at 0.3 and 2.1 hours post start of infusion. As the dosage increased from 50 to 150 mg/kg, and from 150 to 300 mg/kg, the systemic exposure ($AUC_{0-168.083h}$ and C_{max}) to NS101 increased dose-proportionally in both sexes on Day 1 and Day 22. Overall, as the dosage increased from 50 to 300 mg/kg, the systemic exposure ($AUC_{0-168.083h}$ and C_{max}) increased dose-proportionally in both sexes on Day 1 and Day 22. After repeated IV infusion of NS101 at

50, 150, or 300 mg/kg, regarding $AUC_{0\text{-}168.083h}$ change, no marked drug accumulation for NS101 was observed at 150 and 300 mg/kg, but an increase in systemic exposure was observed at 50 mg/kg.[1]

The NOAEL for this study was considered to be 300 mg/kg. The corresponding $AUC_{0\text{-}168.083h}$ and C_{max} of NS101 on Day 22 at NOAEL were 617000000 h*ng/mL and 11600000 ng/mL for males, and 570000000 h*ng/mL and 11000000 ng/mL for females, respectively.[1]

5.2.5 Cross-reactivity Studies in Human Tissues

Cross-reactivity studies indicated that the panel of human tissues was viable. In the tissue titration NS101-FITC positive staining was observed in a variety of cell types and tissue structures. Details are provided in the Investigator's Brochure.[1]

5.2.6 Immunogenicity Studies

In the assessment of immunogenic potential, Neuracle sample NS101 was directly compared to the clinical reference sample ixekizumab (Taltz®) for its ability to induce CD4+ T cell responses. NS101 had a 4% positive response rate in the proliferation assay, suggesting a low risk of clinical immunogenicity. This was lower than the 14% response from the clinical sample ixekizumab. In general, protein therapeutics that induce >10% positive responses in the EpiScreen™ assay would be considered as having an increased immunogenicity risk. Details are provided in the Investigator's Brochure.[1]

The ability of NS101 to induce cytokine secretion in whole blood was assessed in a cohort of 16 healthy human donors, at concentrations ranging from 0.1 µg/mL to 100 µg/mL. Two clinical antibodies, cetuximab (Erbitux®) and alemtuzumab (Lemtrada®), were run in parallel with the samples (Table 8). Cetuximab has a very low incidence of cytokine related infusion reactions in the clinic (~4%) and was used in the assay to set the baseline for absence/rare cytokine release. Alemtuzumab is associated with a very high frequency (~90%) and high concentration of cytokine release in patients. NS101 at concentrations of ≤1 µg/mL showed a similar cytokine profile to cetuximab (details provided in the Investigator's Brochure).[1]

Table 8. Median Cytokine Concentration in pg/mL in Response to Cetuximab (Erbitux®), Alemtuzumab (Lemtrada®) and NS101, at the Indicated Concentrations

CONDITION	Final sample concentration (µg/ml)	IL-6	IL-8	IL-10	IFN-γ	TNF-α
NS101	100	8.7	81.1	14.1	15.2	4.4
	10	5.5	53.0	13.6	13.8	4.2
	1	5.2	33.1	13.3	14.1	4.0
	0.1	4.5	31.4	13.2	14.5	3.6
Erbitux®	100	4.4	53.0	13.4	17.5	4.0
Lemtrada®	10	191.9	537.4	20.0	58.2	36.3

5.3 Summary of Clinical Experience

To date, no human studies have been conducted with NS101.[1]

5.4 Rationale for Study Conduct

Given both AD and ALS are principally disorders of synapse function and nerve connections, novel therapies targeting the deficient synapse circuitry may be beneficial. NS101 could be a transformative breakthrough to treat multiple neurological diseases and is the first therapeutic anti-FAM19A5 antibody entering clinical development.[1]

This study is a FIH, SAD, Phase I study to evaluate the safety, tolerability, PK, PD, and immunogenicity of NS101 in healthy volunteers. It will also aid in the selection of optimal doses and dosing regimens for future studies.

5.4.1 Anticipated Clinical Risks and Benefits

Healthy subjects will not benefit from this study. However, the data from this study may be potentially helpful for future subjects with AD, ALS, or SCI.

Anticipated clinical risks include the following:

- The human-equivalent doses of the two concentrations (10 ug/mL and 100 ug/mL) at which cytokine release may be expected (section 5.2.6) were calculated and found to be 0.375 ~ 3.75 mg/kg in adult males. NS101 in comparison to cetuximab showed slightly higher level of IL-8, and TNF-α; however, the extent of this level was statistically insignificant.[1] For this FIH study, the maximum selected dose is 12 mg/kg, and, from this dose, the expected C_{max} should be approximately 31 ug/mL. Some level of cytokine release may be expected due to the expected C_{max} concentration. With reference to the cytokine profile of NS101 from previous study, we may predict slightly higher level of cytokine release than that of cetuximab.

  Accordingly, infusion-related reactions will be carefully monitored throughout the study.

- Acute reactions following infusion of monoclonal antibodies can be caused by various mechanisms, including acute anaphylactic (IgE-mediated) reactions, serum sickness, tumor lysis syndrome (TLS) and cytokine release syndrome (CRS). Clinical manifestations may include local skin reactions at the injection site, pyrexia and an influenza-like syndrome, to acute anaphylaxis and systemic inflammatory response syndrome. Infusion reactions may occur after NS101 administration.

  This can be managed by recognition of risk factors, appropriate monitoring and prompt intervention. Appropriate hydration and diuresis, premedication and cautious incremental increases in the rate of infusion can minimize risk.

- Lumbar puncture, planned for the current study, may cause headache due to leakage of fluid, pain, bleeding, and brainstem herniation. Subjects will remain at the clinic for a sufficient period of time after the procedure for adequate monitoring.

5.4.2 PK and PD Parameters

NS101 concentrations will be measured in serum for PK evaluation. As PK-PD relationship between NS101 and FAM19A5 has been demonstrated in preclinical studies, FAM19A5 in plasma has been selected as a PD marker for the current study. Furthermore, as presence of NS101 and FAM19A5 has been observed in CSF and is essential for drug efficacy, both NS101 and FAM19A5 will be also measured in CSF after collection via lumbar puncture. In order to mitigate risks associated with lumbar puncture, each subject will be randomized to a single timepoint at which they will undergo lumbar puncture. Furthermore, as lower dose levels that were selected for this study are expected to exhibit no to minimal pharmacological activity, CSF samples will be only collected starting from the medium to high dose levels (Cohorts 5 **to 8**) of the current study. NS101 and FAM19A5 in CSF samples from subjects who will be administered the placebo treatment will be used as baseline.

5.4.3 Rationale for the Study Population

A male healthy volunteer population has been selected for the study because healthy subjects with no concomitant diseases and using no concomitant medications represent a homogenous population allowing for proper evaluation of the safety, tolerability, and PK of a drug without confounding factors.

No reproductive studies have been conducted in NS101. Since risks to a developing foetus or reproductive effects are not known, only male subjects will be included in the study. Furthermore, non-vasectomized male subjects with a female partner of childbearing potential will be required to use an appropriate method of contraception, and will be restricted from sperm donation.

6. Objectives

Primary objective:

To assess the safety and tolerability of NS101 following IV administration of single ascending doses in healthy subjects.

Secondary objectives:

- To characterize the PK profile of NS101 in serum and NS101 concentrations in CSF following single IV infusion doses in healthy subjects.
- To characterize the PD profile of NS101 through FAM19A5 in plasma and FAM19A5 concentrations in CSF following single IV infusion doses in healthy subjects.
- To evaluate the immunogenicity profile of NS101.

7. Study Endpoints

Primary endpoints:

- Incidence, nature, relatedness, and severity of AEs.
- Changes in vital signs measurements, physical examination, clinical laboratory test findings, 12-lead-ECG.

Secondary endpoints:

- Serum PK parameters for NS101 will include: AUC_{0-24}, AUC_{0-t}, AUC_{inf}, C_{max}, T_{max}, $T_{½\ el}$, $K_{el,}$ Cl, and V_z.
- Concentrations of NS101 in CSF.
- Serum to CSF ratio of NS101 concentrations.
- Immunogenicity of NS101, measured as the number and percentage of subjects who develop detectable ADAs and NAbs.

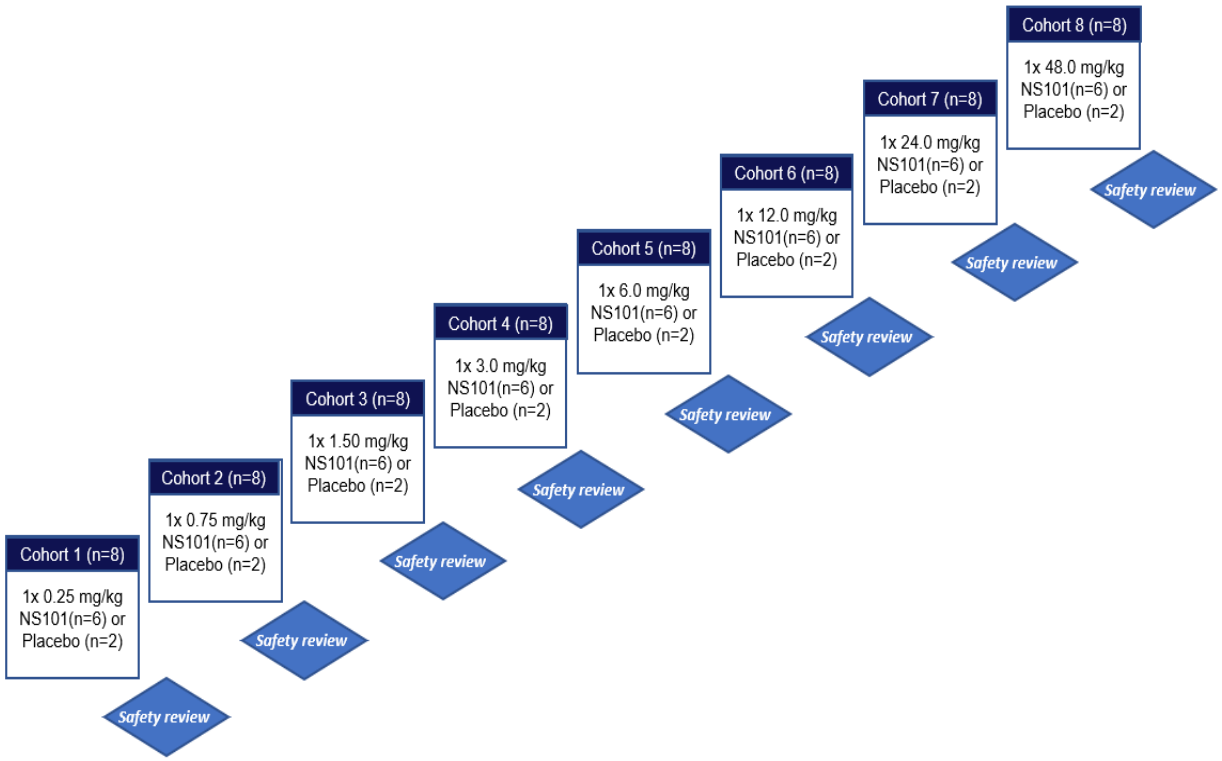
Exploratory endpoints:

- Effect (E): variation of level of FAM19A5 (Δ FAM19A5) following administration of NS101:
    - PD: Change in baseline of FAM19A5 levels
    - PD parameters in plasma: $AUEC_{0-t}$, E_{max}, and TE_{max}.
    - Concentrations of FAM19A5 in CSF.

8. Study Design

This will be a single center, Phase 1, double-blind, randomized, placebo-controlled, sequential SAD study (Figure 9). The study will consist of **8** cohorts (Cohorts 1 to **8**).

Figure 9. Study Diagram



8.1 Design for Cohorts 1 to *8*

The study will evaluate up to **8** single dose levels, each administered in distinct cohorts. Each cohort will include 8 subjects; of these, 6 subjects will receive the active study drug NS101 and 2 will receive matching placebo.

A staggered dosing schedule will be used for each dose level administered under fasting conditions. Two (2) sentinel subjects (1 active and 1 matching placebo) will be dosed first (a minimum of 1 hour apart between end of infusion of first sentinel and start of infusion for second sentinel) and the remaining 6 subjects will be dosed no sooner than 48 hours later (each dosed at least 15 minutes apart).

Cohorts will be dosed sequentially in an ascending fashion, with at least 14 days between the sentinel dosing of each dose level. This period corresponds to approximately one time the half-life of 329.9 hours of NS101 observed in mice and potentially expected in humans.

8.2 Study Progression and Evaluations

A new cohort will not be dosed until the clinical portion of the preceding dose level has been completed, appropriate data has been reviewed by the SRC and authorization to proceed with the next dose level or next study part has been given by the SRC, as described in section 9.

Adjustment to the currently outlined dose levels and period of subjects' follow-up may be made by the SRC based on data from completed dose levels. However, the dose to be administered in a given cohort will not exceed the one currently outlined in the protocol.

8.3 Starting Dose

The calculation of the starting dose for the SAD part of this FIH study was done based on the methodology described in the FDA *Guidance Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers*[2] and considering the recommendations laid down in the EMA *Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products*.[3]

The starting dose was selected based on the totality of the available data from the nonclinical pharmacology, PK and toxicology studies. For determination of the maximum recommended starting dose (MRSD), the following data was considered:

- The NOAEL observed in both rats and Cynomolgus monkeys was 300 mg/kg.[1] However, the important presence of ADAs in Cynomolgus monkeys, in particular, NAbs, does not permit to draw any clear conclusions, since exposure to NS101 may be underestimated. Therefore, PK results from Cynomolgus monkeys were not used for the current FIH study, and their toxicology data was not used to determine the doses or other design elements.

- Rather, the NOAEL value of 300 mg/kg from rats was converted into Human Equivalent Doses (HEDs) by division using a conversion factor of 6.2, and assuming that doses scale 1:1 between animal models and humans, when normalized to body surface area. The HED obtained was 48.4 mg/kg. A potential starting dose of 4.84 mg/kg would provide a dose-based safety margin of 10-fold.

- Additionally, after IV administration of NS101 in a PK-PD study (section 5.2.1.2) at doses ranging from 0.1 to 30 mg/kg in mice, an increase in levels of FAM19A5 appeared at the 0.3 mg/kg dose, which was then identified as the minimally pharmacologically active dose. Mice treated with 10 mg/kg NS101 showed higher FAM19A5 levels 3 days after treatment than 1 day after treatment. FAM19A5 levels 3 days post-treatment with NS101 were similar between the dose of 10 and 30 mg/kg NS101. A dose of 3 mg/kg was the nearest tested dose below the 10 mg/kg dose. Contrary to the higher tested doses, its pharmacological effect decreased on Day 3.[1] Based on these data, a safe pharmacologically active dose (PAD) in mice was established at 3 mg/kg and the HED derived from this PAD is 0.244 mg/kg (rounded up to 0.25 mg/kg). Therefore, a starting dose of 0.25 mg/kg is anticipated to elicit a very low degree of pharmacological activity.

- The selected starting dose of 0.25 mg/kg is lower than the MRSD obtained from the NOAEL from rats (4.84 mg/kg).

8.4 Dose Escalation Scheme

The proposed escalation scheme for the SAD part of the study consists in increments by factors of 3-fold for the first escalation and 2 for all subsequent escalations. As the starting dose of 0.25 mg/kg has been identified as a safe starting dose that is well before the plateau PD phase, it has been judged that decreasing magnitude of incremental factors between each dose escalation is not necessary. Rather, an initial 3-fold escalation from the lowest dose of 0.25 mg/kg, followed by an increase of 2-fold factor for the second escalation, and continuation with the same 2-fold increment for all subsequent escalations was considered appropriate. The planned dose range of 0.25 mg/kg to **48.0** mg/kg was selected to minimize the risk to the subjects while ensuring the evaluation of an exposure range that encompasses and exceeds the systemic exposure where pharmacological effects are anticipated. Based on the favorable safety profile in the toxicology studies, a dose escalation scheme of 3 to 2-fold is not expected to cause any safety concerns in healthy subjects.

Throughout the study, dose levels may be adjusted within the planned dose range based on available safety, tolerability, and PK data from completed cohorts. If a dose level changes from plan, the revised dose will not exceed the one currently outlined in the protocol and subsequent dose increment(s) will not exceed the one(s) currently outlined in the protocol.

The maximum planned dose level of **48.0** mg/kg for the SAD part of the study **does not reach** the HED of 48.4 mg/kg (HED corresponding to a NOAEL of 300 mg/kg). This maximum planned dose should not lead to an exposure that **exceeds 132** 000 000 ng.h/mL, which is the value for $AUC_{0\text{-}24}$ **(or 8** 830 000 ng/mL which is the C_{max}) observed in male rats at 300 mg/kg (established NOAEL) on Day 1 (section 5.2.4.4).

Based on the results of this SAD study, more cohorts and/or additional parts, such as multiple ascending dose evaluations, may be performed. These evaluations would occur only following a protocol amendment.

9. Dose Escalation Criteria

9.1 Data Review by the Safety Review Committee

The SRC will be responsible for the assessment of safety, tolerability, and PK data for each dose level and to make decisions with regards to study progression. The SRC will be composed of at least the Investigator, one medically qualified Sponsor representative and an Independent Medical Monitor designated by CRO.

SRC meetings will be held after safety and tolerability data up to at least Day 8 (for non-sentinel subjects) are available for a minimum of 7 (out of 8) subjects, from a same cohort. PK data up to 48 hours post-dose will also be reviewed to evaluate if PK parameters in humans are as anticipated. The SRC will consider data on a cohort by cohort basis, but also based on cumulative information across cohorts as the study progresses.

The potential decisions of the SRC are:

1) Escalate to the next planned dose level;

2) Continue the study with a more conservative approach, such as slowing the increase in dose for the next dose level or repeating a dose level;
3) Suspend dose escalation until further review of study data can be made, allowing the SRC to determine whether predefined stopping rules have been met. Dose escalation may resume once the SRC concludes that no stopping rules have been met;
4) Stop dose escalation;
5) Terminate the study.

The SRC will conduct a blinded review of the data; however the blind may be broken for individual subjects if judged necessary, prior to making a decision on dose escalation. Dose escalation may occur only following mutual agreement between the SRC members. The SRC may recommend to alter the PK sample collection schedule based on emerging data, including shortening or lengthening the total interval of collection by up to 24 hours, and may add up to 2 additional PK or/and PD samples if necessary. Decisions taken by the SRC will be documented and submitted to the IRB overseeing the study as required (changes in current dosage levels, safety signals), along with supportive data.

A decision to repeat a dose level may be taken to better understand the safety profile before escalating to the next dose level, in the event that AEs occuring on study do not present dose-limiting toxicity, but are present in several subjects.

9.2 Dose Escalation and Stopping Rules

The following sections define stopping rules resulting in an immediate stop to dosing. Each section specifies whether the stop involves a final end of dosing or a temporary halt.

9.2.1 Subject Stopping Rules

Dosing will be permanently stopped for any individual subject experiencing any of the following:

- SAE, regardless of causality to the study drug.
- An AE, physical examination abnormality, ECG abnormality, laboratory parameter abnormality, or vital sign abnormality, possibly or probably related to study drug, and either grade ≥ 3 (Common Terminology Criteria for Adverse Events [CTCAE], current version 5.0) or grade ≥ 2 and considered clinically significant by the SRC.
- Hypersensitivity or anaphylactic reaction related to study drug.
- Medical condition that is judged by the Investigator as to jeopardize the subject's safety if he/she continues to receive the study drug.
- QTc prolongation defined as QTcF increasing ≥ 60 msec and persisting for at least 10 minutes or QTcF > 500 msec and persisting for at least 30 minutes, or episode of torsade de pointes.
- Abnormal liver tests defined as:
    - AST or ALT > 5X upper limit of normal (ULN).
    - AST or ALT > 3X ULN along with one of the following criteria:

- Sustained for more than 2 weeks or
- total bilirubin level > 2X ULN or
- prothrombin time > 1.5X ULN or
- the appearance of fatigue, nausea, vomiting, right upper abdominal pain or tenderness, fever, rash, and/or eosinophilia (> 5%).

- ALP > 3X ULN.
- ALP > 2.5X ULN and total bilirubin > 2X ULN.
- ALP > 2.5 ULN with the appearance of fatigue, nausea, vomiting, right upper abdominal pain or tenderness, fever, rash, and/or eosinophilia (> 5%).

9.2.2 Cohort Stopping Rules

9.2.2.1 Stopping Rules After Sentinel Dosing

The following events occurring in one sentinel subject will result in a temporary halt of dosing within that cohort:

- The occurrence of one SAE considered at least possibly related to the study drug.
- The occurrence of a CTCAE grade 3 considered at least possibly related to the study drug.

If any the above stopping rules is met, the remaining subjects of the cohort will not be dosed until the SRC reviews details of the event and/or additional data. If, after further review of the data, the SRC determines that no stopping rules have been met (e.g., AE determined to be unrelated to the study drug), then dosing of the remaining subjects of the cohort may proceed.

9.2.3 Stopping Rules for Dose Escalation

Dose escalation will be temporary halted if the SRC suspects that any of the stopping rules described in sections 9.2.3.1 and 9.2.3.2 is met. Continuation or initiation of cohorts at lower dose levels will be evaluated on a case-by-case basis. If, after further review of the study data, it is determined by the SRC that no stopping rules have been met (e.g., AE determined to be unrelated to the study drug), then dose escalation may resume. However, if the SRC confirms that a stopping rule has been met, dose escalation will be stopped definitely for that part of the study.

9.2.3.1 Events Within One Dose Level

Dose escalation will be halted if any of the events described below occur within one dose level:

- Individual stopping rules met for ≥ 2 subjects in the same cohort suggesting that subjects receiving higher dose levels would be at risk for similar adverse drug reactions.
- The occurrence of one SAE considered at least possibly related to the study drug.
- The occurrence of CTCAE grade 3 considered at least possibly related to the study drug in ≥ 2 subjects and independent of within or not within the same system organ class (SOC).
- Clinically significant abnormalities of the same character, at least of CTCAE grade 2, and considered at least possibly related to the study drug in ≥ 2 subjects, with regards to:

- the physical examination;
- ECG;
- vital signs; or
- clinical laboratory parameters.

- Mean exposure (AUC_{0-24}) exceeding 132 000 000 **ng.h/mL or concentrations** reaching 8 830 000 ng/mL.

9.2.3.2 Cumulative Study Events

In addition to the events listed in section 9.2.3.1, any of the events described below occurring at different dose levels administered throughout the study (i.e., cumulative events) will also result in a temporary halt of dose escalation:

- The occurrence of CTCAE grade 3 of the same character considered at least possibly related to the study drug in ≥ 2 subjects.
- Clinically significant abnormalities of the same character, at least of CTCAE grade 2, and considered at least possibly related to the study drug in ≥ 3 subjects, with regards to:
  - the physical examination;
  - ECG;
  - vital signs; or
  - clinical laboratory parameters.
- Determination by the SRC that a pattern of AEs precludes further dose escalation, even if no other stopping rules have been met.

9.2.4 Study Stopping Rules

The study will be terminated if any of the following criteria are met:

- The occurrence SAEs considered at least possibly related to the study drug in ≥ 2 subjects and independent of within or not within the same SOC.
- If ≥ 2 subjects develop the same CTCAE grade 3 or if 1 subject develops a CTCAE grade 4 or higher, considered at least possibly related to the study drug.
- Determination by the SRC that a pattern of AEs precludes any further dosing.

10. Study Population

10.1 Sample Size

It is planned to enroll up to **80** male volunteers for participation in this study.

The study will consist of **8** cohorts, each including 8 subjects (6 subjects receiving the study drug NS101 and 2 receiving matching placebo), for a total of **64** subjects planned for evaluation.

Subjects who withdraw or are withdrawn from the study after dosing, for reasons other than safety and tolerability, may be replaced after consultation between the SRC members. The total number

of subjects dosed (including potential replacement subjects) will remain within a maximum of 10 subjects per cohort.

The sample size of this study is not determined based on statistical calculations, it is rather determined based on the probability of observing an AE. A sample size of 8 subjects per cohort randomized in a 3:1 ratio to the study drug versus placebo represents a typical panel for a FIH study. This number of subjects is judged adequate to achieve the study objectives. Therefore, up to **80** healthy adult male volunteers will be dosed.

10.2 Inclusion Criteria

Subjects enrolled in this study will be members of the community at large. The recruitment advertisements may use various media types (e.g., radio, newspaper, the clinical site Web site and volunteer database). Subjects must meet all of the following criteria to be included in the study:

1) Male, non-smokers (no use of tobacco or nicotine products within 6 months prior to screening), ≥18 and ≤55 years of age, with BMI >18.5 and <30.0 kg/m^2 and body weight ≥50.0 kg for males.
2) Healthy as defined by:
    a) the absence of clinically significant illness and surgery within 4 weeks prior to dosing.
    b) the absence of clinically significant history of neurological, endocrine, cardiovascular, respiratory, hematological, immunological, psychiatric, gastrointestinal, renal, hepatic, and metabolic disease.
3) Subject's score on the S-STS at screening must be 0.
4) Male subjects who are not vasectomized for at least 3 months prior to dosing, and who are sexually active with a female partner of childbearing potential (childbearing potential females are defined as women that are neither post-menopausal nor surgically sterile) must be willing to use one of the following acceptable contraceptive methods from the study treatment infusion and for 90 days after.
    a) simultaneous use of condom and hormonal contraceptive used for at least 4 weeks or intrauterine contraceptive device placed for at least 4 weeks for the female partner;
    b) simultaneous use of condom with spermicide and a diaphragm or cervical cap for the female partner.
5) Male subjects (including men who have had a vasectomy) with a pregnant partner must agree to use a condom from the study treatment infusion and for 90 days after.
6) Male subjects must be willing not to donate sperm for 90 days after study treatment infusion.
7) Capable of consent.

10.3 Exclusion Criteria

Subjects to whom any of the following applies will be excluded from the study:

1) Any clinically significant abnormality at physical examination, clinically significant abnormal laboratory test results or positive test for HIV, hepatitis B, or hepatitis C found during medical screening.
2) Positive urine drug screen or alcohol breath test at screening or admission.
3) History of asthma, urticaria, anaphylactic reactions, or any other clinically significant allergic reactions to any medication, including biologics, or food, or allergy to any excipient in the formulation.
4) Clinically significant ECG abnormalities (QTcF >450 ms) or vital sign abnormalities (systolic BP lower than 90 or over 140 mmHg, diastolic BP lower than 50 or over 90 mmHg, HR less than 50 or over 100 bpm) at screening.
5) History of alcohol abuse within 1 year prior to screening or regular use of alcohol within 6 months prior to screening that exceeds 14 units of alcohol per week (1 unit = 150 mL of wine, 360 mL of beer, or 45 mL of 40% alcohol) or 3 to 4 units per day.
6) History of drug abuse within 1 year prior to screening or recreational use of soft drugs (such as marijuana) within 1 month or hard drugs (such as cocaine, PCP, crack, opioid derivatives including heroin, and amphetamine derivatives) within 3 months prior to screening.
7) Participation in a clinical research study involving the administration of an investigational or marketed drug or device within 30 days prior to study treatment infusion, administration of a biological product in the context of a clinical research study within 90 days prior to study treatment infusion, or concomitant participation in an investigational study involving no drug or device administration.
8) Use of medications for the timeframes specified below, with the exception of medications exempted by the Investigator on a case-by-case basis because they are judged unlikely to affect the PK profile of the study drug or subject safety (e.g., topical drug products without significant systemic absorption):
    a) prescription medications within 14 days prior to study treatment infusion;
    b) any vaccination, including COVID-19 vaccine, within 14 days prior to study treatment infusion;
    c) over-the-counter products and natural health products (including herbal remedies such as St. John’s wort, homeopathic and traditional medicines, probiotics, food supplements such as vitamins, minerals, amino acids, essential fatty acids, and protein supplements used in sports) within 7 days prior to study treatment infusion, with the exception of the occasional use of acetaminophen (up to 2 g daily);
    d) depot injection or implant of any drug within 3 months prior to study treatment infusion.
9) Receiving treatment or participation in an organized weight loss program that may cause significant weight gain or loss within 1 month before dosing.
10) Donation of plasma or serum within 7 days prior to dosing. Donation or loss of blood (excluding volume drawn at screening) of 50 mL to 499 mL of blood within 30 days, or more than 499 mL within 56 days prior to study treatment infusion.

11) History of blood dyscrasias, including, but not limited to, thrombocytopenia, thrombocythaemia, or arterial/venous thromboembolic complications.

12) History of lymphatic disorders.

13) History of hypertriglyceridemia.

14) Current or history of malignancy.

15) Significant history of seizures, prior traumatic brain injury, schizophrenia, schizoaffective disorder, or bipolar disorder.

16) Any reason which, in the opinion of the Investigator, would prevent the subject from participating in the study.

17) For subjects in cohorts that will include a lumbar puncture (Cohorts 5 **to 8**): Medical conditions in which a lumbar puncture is contraindicated, including coagulopathy, thrombocytopenia, prior lumbar spinal surgery, or other factor that precludes safe lumbar puncture in the opinion of the Investigator.

18) For subjects in cohorts that will include a lumbar puncture (Cohorts 5 **to 8**): hypersensitivity to anesthetics, such as lidocaine, or any of its components.

11. Clinical Procedures

Unless otherwise specified, procedures, data collection and evaluation will be conducted as per the clinical site SOPs.

11.1 Screening Procedures

Subject screening procedures will be performed within 28 days preceding administration of study medication. Subjects must provide written informed consent prior to initiation of any screening procedures. The consent to perform some general screening procedures may be obtained on a consent document other than the Informed Consent Form (ICF) specific to this study, and therefore, some screening test results could be obtained before signature of the ICF specific to this study. The study-specific ICF must be signed and dated by the subject before participation to study-specific procedures.

Screening procedures will include: demographic data (age, gender, and handedness), medical and medication histories, complete physical examination, body measurements (height, weight, and BMI), ECG (12-lead), vital signs (BP, HR, RR, and OT), S-STS, hematology, biochemistry, coagulation, serology (HIV, hepatitis B and C tests), urinalysis, alcohol breath test, and urine drug screen.

For eligibility purposes, abnormal laboratory or vital signs results may be repeated once if abnormal result is observed at the initial reading. Moreover, abnormalities found in the ECG may need to be confirmed by repeated measurements. In the event that the participation of a subject in the study is delayed and some screening procedures had been performed outside of the prescribed screening window, outdated screening procedures can be repeated.

11.2 Confinements and Visits

For all cohorts, subjects will be confined from the day before dosing (Day -1) until after the 48-hour post-start of infusion blood draw. Subjects will come back to the clinical site for all subsequent study assessments on Days 5±1, 8±1, 15±1, 22 ±2, 29 ±2, and 60 ±3 (study exit or ET).

Participation of each subject in this study should last approximately 2 months.

11.3 Randomization and Blinding

Subjects will be administered each treatment (NS101 or placebo), according to the block randomization scheme. The subjects and the clinical personnel involved in the collection, monitoring, revision, or evaluation of AEs, or personnel who could have an impact on the outcome of the study will be blinded with respect to the subject's treatment assignment (NS101 or placebo). Blinding will be maintained at least until the clinical phase of the study is completed (i.e., when reporting and evaluation of all AEs have been completed, for all cohorts).

Designated pharmacy personnel at the clinical site not directly involved with the clinical aspects of the trial will prepare and dispense the study medication and will be aware of the randomization code. The study drug and placebo will have the same visual appearance in order to avoid compromising the study blinding.

A staggered dosing schedule will be used for each cohort and will include 2 sentinel subjects randomized in a 1:1 ratio (1 active and 1 placebo) dosed initially, and the remaining 6 subjects (5 active and 1 placebo) will be dosed at least 48 hours later.

One randomization scheme will be produced for each cohort separately.

Within each cohort in Cohorts 5 **to 8**, subjects will be randomized a second time to one (1) unique timepoint for collection of a CSF sample (via lumbar puncture). Each of the 4 timepoints specified in section 11.8.3 will be assigned to 2 subjects. Sentinel subjects will be randomized to the same timepoint, to ensure that each timepoint is assigned to at least 1 subject that is on active drug. Although lumbar puncture is planned starting from Cohort 5, cumulative safety/tolerability data from previous cohorts will inform on whether lumbar puncture will be started at Cohort #5.

In the event of an emergency for an individual subject in which knowledge of the study treatment is critical to the subject's medical management or for the decision of dose escalation, the Investigator may break the blind for that subject. An envelope for each subject containing the treatment assignment will be available from the pharmacy personnel. The Investigator or other attending study physician will make every effort to contact the Sponsor prior to unblinding a subject's treatment assignment and will record the date and reason for unblinding in the study source documents.

As all samples (including placebos) will be analyzed, the randomization code will not be available to the Bioanalytical Division of Syneos Health prior to analysis from each cohort. However, since concentrations of NS101 above the analytical method's lower limit of quantification (LLOQ) should not be observed for subjects receiving the placebo, blinding of treatment assignments by personnel in the Bioanalytical Division involved with sample analysis cannot be assured completely.

Blinded PK results will be available during the course of the study. These results will be reported without revealing the subject's identity.

11.4 Investigational Products

Active ingredient, NS101: NS101 solution for injection in glass vial and developed in 400 mg/16 mL (25 mg/mL) strength, Manufactured by BINEX Co., Ltd. and supplied in 20 mL glass injection vials (Neuracle Science Co., LTD., Republic of Korea) Details in Table 9 and Table 10.

Placebo for NS101: Matching placebo solution without the active component, composed of the same ingredients as NS101 solution for IV injection, with the same visual appearance once diluted. To be supplied in 20 mL glass injection vials. Manufactured by BINEX Co., Ltd. and supplied in 20 mL glass injection vials (Neuracle Science Co., LTD., Republic of Korea) Details in Table 11.

Table 9. List of General Physicochemical, Biological and Immunochemical Properties of NS101

Appearance	Clear, colorless or yellowish solution
Molecular Weight	~146 kDa
pI of main band	Theoretical pI: 5.56, IEF gel: pI 4.5 – 7.4
Number of amino acids	Heavy chain (HC): 461 amino acids Light chain (LC): 214 amino acids
Polypeptide chain composition	Two (2) hetero dimers, each comprising an HC and LC
Disulfide bonds	Four (4) intra-chain LC disulfide bonds; two per light LC Ten (10) intra-chain HC disulfide bonds: five per HC Two (2) inter-chain disulfide bonds between the two HC Two (2) inter-chain disulfide bonds; one each between HC and LC
Secondary structure (by circular dichroism analysis)	α-helix: 6.8% β-sheet (anti-parallel and parallel): 47.9 – 48.5% β-turn: 14.8% random coil: 32.1 – 32.4%
Potency (ELISA)	70-130%
Binding specificity	Specific binding to FAM19A5

Table 10. Composition of NS101

Component	Quantity per Vial	Final Concentration	Function	Quality Standard
NS101 Active substance	400 mg	25 mg/mL	Active substance	In-house
L-Histidine-HCl	24.824 mg	10 mM	Buffer	EP

Hydrochloric acid	q.s.	---	pH modifier	EP
Polysorbate 20	1.6 mg	0.01%	Surfactant	EP
Sodium chloride	140.256 mg	150 mM	Isotonic agent	EP
Water for injection	q. s. to 16.0 mL	---	Solvent	EP

Table 11. Composition of Placebo for NS101

NS101 Placebo is a sterile preservative-free solution for IV infusion in colorless glass vial (USP type I glass vial, 20mL). A 20 mL glass vial contains 16 mL of NS101 Placebo solution for injection.

Component	Quantity/vial	Function	Specification
L-Histidine	24.824 mg	Buffer	EP
Hydrochloric acid	q.s.	pH modifier	EP
Polysorbate 20	1.6 mg	Surfactant	EP
Sodium chloride	140.256 mg	Isotonic agent	EP
Water for injection	q.s. to 16.0 mL	Solvent	EP

11.5 Drug Supplies and Accountability

It is the responsibility of the Sponsor to ensure that study medication provided for this study are manufactured under Good Manufacturing Practice (GMP) and are suitable for human use. The Sponsor is responsible to ship a sufficient amount of dosage units to allow the clinical site to maintain an appropriate sampling for the study.

Study medication will be stored at the clinical site as per applicable requirements. The medications will be stored in a locked, environmentally-controlled medication room with restricted access. Container(s) will bear a label containing at least the name of the study drug, lot and/or batch number, and manufacturing and expiry date.

Individual doses for each subject will be dispensed at the clinical site, as per appropriate SOP. Individual doses will be dispensed according to the randomization scheme in appropriate dosing containers indicated with at least the project number, the cohort number and the subject number/spare number.

All study drug received at the site will be inventoried and accounted for throughout the study and the result recorded in the drug accountability/retention record according to the clinical site appropriate SOP**.** At the end of the study, used vials will be destroyed at the clinical site as per clinical site SOP.

NS101 and placebo for NS101 should be refrigerated at 2-8°C and should not be frozen. NS101 was stable for 18 months without any significant changes in quality. NS101 is being monitored for stability at the long-term storage condition of 5±3°C for 36 months.[1]

11.6 Study Treatments Administration

The study treatment will be administered in bed by IV infusion using aseptic techniques. Detailed instructions for study treatment infusion will be included in Pharmacy forms and/or Project Specific Procedure (PSP).

The body weight of each subject measured on Day -1 (check-in) will be used to calculate the exact individual dose required on a mg/kg basis, based on their assigned dose level (cohort), as presented in Table 12. The infusion will be performed over a period of approximately 60 minutes at a constant rate using a volume-controlled infusion device.

Table 12. Dose Levels per Cohort

Cohort	Dose levels of NS101*
1	1 x 0.25 mg/kg
2	1 x 0.75 mg/kg
3	1 x 1.50 mg/kg
4	1 x 3.0 mg/kg
5	1 x 6.0 mg/kg
6	1 x 12.0 mg/kg
7	**1 x 24.0 mg/kg**
8	**1 x 48.0 mg/kg**

* Volume of NS101 placebo will be also determined based on subject weight and NS101 concentration.

As an example, for a human weighing 60 kg assigned to the first dose level of 0.25 mg/kg, the administered dose would be equal to 15 mg.

Approximately 1 hour prior to start of study drug infusion, an IV port will be inserted into the antecubital region and a sterile normal saline solution infusion will be initiated at fixed rate in order to keep the vein open. The study drug will be infused over approximately 60 minutes at a constant rate. At the end of the infusion, 3 mL of saline solution will be injected to flush the remaining drug into the IV catheter. The end of infusion will be set to the end of the 3 mL flush. For safety reason (e.g., administration of rescue medication), the IV line will remain opened for approximately 1 hour following completion of infusion.

The start and end times of infusion of each subject will be recorded in the source documents. Time of dosing will be set as the start of study treatment infusion.

Diluted NS101 should be used within 4 hours at room temperature. Diluted NS101 stored at 2ºC to 8ºC should be equilibrated to room temperature for 30 minutes prior to infusion.

11.7 Study Restrictions

11.7.1 Food and Fluids

For standardization purposes, no food will be allowed from at least 8 hours prior to start of infusion until at least 2 hours after the start of study drug infusion. A light meal will be served more than 2 hours after the start of study treatment infusion.

Water will be provided *ad libitum* at all times.

In addition, subjects will be required to abstain from:

- food containing poppy seeds within 24 hours prior to admission;
- food or beverages containing xanthine derivatives or xanthine-related compounds or energy drinks from 48 hours prior to dosing and during confinement.

11.7.2 Tobacco, Alcohol, and Illicit Drugs

Subjects will be required to abstain from using soft or hard drugs or any tobacco or nicotine products from screening and throughout the study.

Consumption of alcohol-based products will be prohibited from 24 hours prior to admission, and 24 hours prior to any study assessment visit.

11.7.3 Concomitant Medications

Subjects will be required to avoid using prescription and over-the-counter medications for the period of time specified in exclusion criterion #8) and throughout the study. Subjects will be required to avoid using natural health products (including herbal remedies, homeopathic and traditional medicines, probiotics, food supplements such as vitamins, minerals, amino acids, essential fatty acids, and protein supplements used in sports) from 7 days prior to dosing and throughout the study.

No concomitant medications are allowed during the study, with the exception of one(s) required for the medical management of an AE, medications exempted by the Investigator on a case-by-case basis that are judged unlikely to affect the PK profile of the study drug or subject safety (e.g., topical drug products without significant systemic absorption) and occasional use of acetaminophen.

Subjects will also be required to avoid receiving any vaccination, including COVID-19 vaccine, from 14 days prior to dosing and throughout the study. If vaccination is required for any reason, it must first be discussed with and exempted by the Investigator on a case-by-case basis to ensure that it does not compromise the PK profile of the study drug or the subject safety.

All medications taken by subjects after screening until the last study day will be documented as concomitant medications. Any concomitant medication use, other than the allowed medications stated above, will be reviewed and evaluated on a case-by-case basis by the Investigator to

determine if they affect a subject's eligibility or continued participation in the study, or for potential impact on the study results.

11.7.4 Posture and Physical Activity

For safety reasons, subjects will be required to remain supine and avoid sleeping from approximately 1 hour before start of infusion until after the first 4 hours after start of study drug infusion.

However, failure of subjects to comply with these requirements does not constitute a deviation from the protocol if it is medically necessary, required for procedures, or to go to the bathroom. When appropriate, subjects will be accompanied by a staff member while walking. Vigorous activity will be prohibited at all times during the confinement.

11.7.5 Other Restrictions

Only for cohorts undergoing lumbar puncture (Cohorts 5 **to 8**), subjects will be asked not to wear artificial nails or nail polish on days of pulse oximetry during lumbar puncture (Days 2, 8, and 15), at least on the fingers used for PO monitoring, in order to avoid false readings.

11.8 Sample Collection and Processing

11.8.1 Blood Sampling for PK Analysis

A total of 21 blood samples will be collected in each cohort for PK analysis: pre-start of infusion and 0.25 (±3 min), 0.5 (±3 min), 0.75 (±3 min), 1 (±3 min), 1.25 (±3 min), 1.5 (±3 min), 2 (±3 min), 4 (±3 min), 6 (±3 min), 8 (±3 min), 12 (±15 min), 24 (±15 min) (Day 2), 36 (±15 min) (Day 2), 48 (±15min) (Day 3), 96 (Day 5±1), 168 (Day 8±1), 336 (Day 15±1), 504 (Day 22±2), 672 (Day 29±2), and 1416 (Day 60±3) hours post-start of infusion (3 mL for each sampling time).

11.8.2 Blood Sampling for PD Analysis

A total of 14 blood samples will be collected in each cohort for PD analysis: pre-dose and 1, 4, 6, 12, 24 (Day 2), 36 (Day 2), 48 (Day 3), 96 (Day 5±1), 168 (Day 8±1), 336 (Day 15±1), 504 (Day 22±2), 672 (Day 29±2), and 1416 (Day 60±3) hours post-start of infusion (3 mL for each sampling time).

11.8.3 Lumbar Puncture for CSF Sampling

For Cohorts 5 **to 8** only: for each subject, a single CSF sample will be collected via lumbar puncture for PK and PD analysis. The volume of collected CSF per sample should not exceed approximately 6 mL.

Subjects in each cohort will be randomized to have their CSF sample collected at one of the following timepoints, so as to have 2 subjects assigned to each timepoint: 24 (±3 hours; Day 2), 36 (±3 hours; Day 2), 168 (Day 8±1), or 336 (Day 15±1) hours post-start of infusion, according to

the randomization scheme. Sentinel subjects will be randomized to the same timepoint. Placebo subjects will serve to evaluate change from baseline.

Lumbar puncture may require administration of a local anesthetic, such as lidocaine. Details would be described in a separate document.

A catheter will be inserted during the procedure for safety.

Following the lumbar puncture, subjects could be observed for up to 6 hours while in supine position. The Investigator or designee will then assess the subject to determine whether it is safe for them to leave the clinic. If there is a safety concern, the Investigator or designee can request the subject to remain for further monitoring as needed. Subjects should refrain from physically strenuous activities for 12 hours after lumbar puncture and may then resume their usual routines.

Vital signs will be also measured before lumbar puncture and will be measured approximately 0.5 hours after lumbar puncture, meaning approximately 24.5 (±3 hours; Day 2), 36.5 (±3 hours; Day 2), 168.5 (Day 8±1), or 336.5 (Day 15±1) hours post-start of infusion, according to the randomization scheme.

PO will be performed during lumbar puncture: 24 (±3 hours; Day 2), 36 (±3 hours; Day 2), 168 (Day 8±1), or 336 (Day 15±1) hours post-start of infusion, according to the randomization scheme.

Details will be included in the PSP.

Cumulative safety/tolerability data from early cohorts will inform on whether lumbar puncture will be started at Cohort 5.

11.8.4 Anti-Drug Antibody Measurement (Immunogencity)

A total of 6 immunogenicity blood samples will be collected for ADA and NAbs: pre-start of infusion and 96 (Day 5±1), 336 (Days 15±1), 504 (Day 22±2), 672 (Day 29±2), and 1416 (Day 60±3) hours post-start of infusion (3 mL for each sampling time).

11.8.5 Time Tolerance Windows and Total Blood Volume

The time tolerance window for blood samples collected during the confinement period will be within the hour pre-dose for the pre-infusion sample, ±3 minute for all samples collected before 8 hours post-start of infusion and ±15 minutes for all subsequent samples collected up to 48 hours post-start of infusion. The time tolerance window for return visit samples will be ±1 day on Days 5, 8 and 15, ± 2 days for Days 22 and 29, and ± 3 days for Day 60. The time tolerance window for CSF samples on Day 2 is ± 3 hours. Sample collections done outside the pre-defined time windows will not be considered as protocol deviations since actual post-start of infusion sampling times will be used for PK and statistical analyses. Unless otherwise specified or for subject safety, when blood draws and other procedures coincide, blood draws will have precedence. When timepoints for ECGs, vital signs, and blood draws coincide, procedures will be carried out in the said order. A dead-volume intravenous catheter will be used for blood collection to avoid multiple skin punctures, when appropriate. Otherwise, blood samples will be collected by direct venipuncture.

The planned volume of blood to be collected during the study, including that collected for eligibility and safety purposes, should not exceed 250 mL. Additional tests or blood draws could be performed, if deemed required by the Investigator or study staff.

Serum and CSF samples will be collected and processed as per the Analytical Methodology Information Sheet / Laboratory Manual.

11.9 Subject Monitoring

Subjects will be monitored throughout the study by the clinical staff for AEs. In each cohort, the Investigator or designee will be on site one hour prior to the start of treatment infusion and until 5 hours post-start of infusion, and available on call for the remainder of the study. If necessary, the Investigator or designee at the clinical site or a healthcare professional in a nearby hospital will administer treatment for any AE(s). A crash cart or emergency bag containing the necessary rescue material and appropriate medications will be available in the clinic to allow rapid intervention in case of emergency.

Safety parameters, including laboratory results and ECG, will be assessed by the Investigator or designee, using the clinical site's criteria for biomedical laboratory and ECG acceptance ranges as suggested guidelines in making the medical assessment.

Scheduled safety measurements will be repeated according to the clinical site SOPs or upon request from the Investigator or designee. Any abnormal repeated measurement will be evaluated by the Investigator or designee and repeated if judged necessary. Further action may be taken upon the Investigator or designee’s request.

Subjects will be advised to notify their healthcare professional(s) (e.g., physician, dentist, and/or pharmacist) that they are participating in a clinical research study on a drug being developed by Neuracle Science Co., LTD. for the potential clinical use in adult patients with neurodegenerative diseases, including AD, ALS, and SCI, called NS101, before taking any medicines or undergoing any medical procedure.

11.9.1 Vital Signs

BP, HR, RR, and OT will be measured in a sitting position (except for safety reasons) at screening and at study exit.

BP, HR, RR, and OT will also be measured on Day -1. BP, HR, and OT will also be measured on Day 1 pre-infusion and 1.5, 2, 4, 6, 8, 12, 24 (Day 2) and 48 (Day 3) hours post-start of infusion, and on Days 5 ±1, 8±1, 15±1, 22±2, and 29±2. BP, HR, RR, and OT will be measured in a supine position during confinement.

When vital signs measurements coincide with a blood draw, they should preferably be performed before the blood collection whenever possible.

11.9.2 12-lead ECG

Supine 12-lead ECG will be performed at screening and study exit.

Supine ECG will also be performed on Day 1 pre-infusion and 1.5, 4, 8, 12, 24 (Day 2), and 48 (Day 3) hours post-start of infusion, and on Days 5±1, 8±1, 15±1, 22±2, and 29±2.

When ECG coincides with a blood draw, it should preferably be performed before the blood collection whenever possible.

11.9.3 Physical Examination

A complete physical examination will be performed at screening and study exit. A complete physical examination includes assessments of the following: head, eyes, ears, nose, throat (HEENT), neck, chest, lungs, abdomen, musculoskeletal, dermatological, cardiovascular/peripheral vascular, and general neurological examination.

A brief physical examination will be done on Day -1, Day 3 (48 hours post-start of infusion), Day 8±1, and Day 15±1, and as directed by symptoms.

A brief physical examination includes assessments of the following: HEENT, chest, lungs, abdomen, dermatological, cardiovascular/peripheral vascular, and areas of note elicited from the subject.

11.9.4 Drug and Alcohol Screen

A urine drug screen (amphetamines, methamphetamines, barbiturates, benzodiazepines, tetrahydrocannabinol, cocaine, opiates, PCP, MDMA, methadone) and an alcohol breath test will be performed at screening and at admission on Day -1.

11.9.5 Sheehan Suicidality Tracking Scale (S-STS)

The S-STS will be performed at screening and study exit. The S-STS is a prospective, patient self-reported or clinician administered rating scale that contains 16 questions to track both treatment-emergent suicidal ideation and behaviors.[4,5]

This scale will be clinician administered, completed on site, and will be in paper format. The source document will be provided by the Sponsor. The assessment period for completing the scale will be lifetime look back prior to screening for the assessment at the screening visit and since screening for the assessment at the study exit visit.

If the Investigator determines that a subject is at risk of suicide or self-harm, appropriate measures to ensure the subject's safety and obtain mental health evaluation must be implemented. The subject must immediately be discontinued from the study. The event should be recorded as either an AE or a serious AE (SAE) as determind by the Investigator and reported within 24 hours to the Sponsor.

11.9.6 Laboratory Assessments

11.9.6.1 Hematology

Hematology will be performed at screening and study exit.

Hematology will also be performed on Day -1, Day 3 (approximately 48 hours post-start of infusion), and Days 8±1, 15±1, and 29±2.

The following will be assessed: complete blood count with differential, hemoglobin, and hematocrit.

11.9.6.2 Biochemistry

Biochemistry will be performed at screening and study exit.

Biochemistry will also be performed on Day -1, Day 3 (approximately 48 hours post-start of infusion), and Days 8±1, 15±1, and 29±2.

The following will be assessed: albumin, alkaline phosphatase, AST, ALT, urea, calcium, chloride, glucose, phosphorus, potassium, creatinine, sodium, total bilirubin, total protein, total cholesterol, HDL, LDL, and triglycerides.

11.9.6.3 Coagulation

Coagulation will be performed at screening and study exit.

Coagulation will also be performed on Day -1, Day 3 (approximately 48 hours post-start of infusion), and Days 8±1, 15±1, and 29±2.

The following will be assessed: prothrombin time (PT)/ international normalized ratio (INR) and activated partial thromboplastin time (aPTT).

11.9.6.4 Serology

Serology will be performed at screening. The following will be assessed: HIV antigen and antibody, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV) antibody.

11.9.6.5 Urinalysis

Urinalysis will be performed at screening and at study exit.

Urinalysis will also be performed on Day -1, Day 3 (approximately 48 hours post-start of infusion), and Days 8±1, 15±1, and 29±2.

The following will be assessed: macroscopic examination, pH, specific gravity, protein, glucose, ketones, bilirubin, occult blood, nitrite, urobilinogen, and leukocytes. Unless otherwise specified, microscopic examination will be performed according to internal procedures.

11.9.7 Injection Site evaluation

Skin reactions at the injection site will be recorded prior to start of study drug infusion and approximately 0.5, 1, 24 (Day 2), 48 (Day 3), 96 (Day 5±1) and 168 (Day 8±1) hours post-start of infusion.

A trained observer, whom is blinded to the randomization scheme, will check and record local dermal reaction at the application site using the rating scale in Table 13. When possible, the same evaluator will follow the subject through the study. In the event that more than one evaluator is required to follow the subject through the study, every precaution should be taken to limit the number of evaluators.

Table 13. Injection Site Reaction Scores

None	0	No reaction
Mild	1	Easily tolerated erythema and/or light bruising and/or mild pain
Moderate	2	Distributing erythema with swelling and/or distributing bruising and/or distributing pain
Severe	3	Almost intolerable symptoms, or clinically definite skin necrosis, characterized by any of the following: oozing, weeping, skin breakdown, ulceration, scar formation

In addition, bleeding or fluid loss at the injection site will be documented. The diameter of any erythema, swelling or induration will be measured (mm) in two dimensions.

Reactions < 10 mm will not be listed as an AE unless it persists for more than 24 hours.

11.10 Management of Infusion-Related Reactions and Other Emergency Events

Subjects will be closely monitored by a medical team during drug infusion and in the hours following the end of infusion, for the presence of symptoms of infusion-related reactions (IRR). Subjects will be also encouraged to quickly report any symptoms at any time they occur during the confinement.

If a subject experiences an IRR during study drug infusion, the infusion should be stopped and the subject's condition should be evaluated by a physician. The physician may decide to restart the infusion if the signs and symptoms have resolved. If the infusion is slowed, the total infusion time should not exceed 3 hours (± 10 minutes). The study drug should be stopped if the infusion reaction recurs.

The necessary rescue material, equipment and appropriate medications will be available in the clinic to allow rapid intervention in case of emergency including, but not limited to:

- oxygen
- epinephrine for i.v. or endotracheal injection
- antihistamines

- corticosteroids
- i.v. infusion solutions, tubing, catheters, and tape

The following procedures will be followed in the event of a suspected anaphylactic reaction during study drug infusion:

- Stop the study drug infusion.
- Disconnect from the cannula and aspirate 10 mL blood from the dosing cannula if the infusion is to be ceased immediately.
- Maintain an adequate airway.
- Administer antihistamines, epinephrine, or other medications as required by subject status and directed by the Investigator.
- Continue to observe the subject and document observations.

11.11 Study Exit Procedures and ET

Complete physical examination, hematology, biochemistry, coagulation, urinalysis, vital signs (BP, HR, RR, and OT), S-STS, ECG, and AE monitoring, as well as collection of PK, PD, and immunogenicity samples, will be performed on the last study visit (Day 60 ±3). If not possible, all efforts will be made to complete study exit procedures within 14 days after the last participation of the subject in the study.

11.12 Data Collection and Evaluation

The electronic source data capture system is the primary data collection instrument for the study. Collection screen in the electronic source data capture system will be utilized for the collection of all data. Data will be entered using the English language and should be kept current to enable the monitor to review the subjects' status throughout the course of the study.

Source data should be ALCOA-C (attributable, legible, contemporaneous, original, accurate, and complete). Source documents will be maintained in order to maintain data integrity. The Investigator and/or the clinical staff have the responsibility of ensuring the accuracy, completeness, legibility, and timeliness of the source data.

Details on the data management process will be described in a data management plan (DMP).

11.13 Subject Withdrawal and Replacement

Subjects will be advised that they are free to withdraw from the study at any time. Over the course of the study, the Sponsor and the Investigator or designee may withdraw any subject from the study for one of the reasons described below; subject withdrawal will be done in accordance with the clinical site's SOP:

- Safety reason;
- Non-compliance with protocol requirements;
- Significant protocol deviation;

- Positive alcohol breath test, or drug screen.

Hematology, biochemistry, coagulation, and urinalysis results will be reviewed by the Investigator or designee prior to the infusion; subjects will be withdrawn from the study if it is deemed that the subject's safety may be at risk on the basis of these test results.

Subjects who withdraw, or are withdrawn, from the study for safety and tolerability reasons after dosing will not be replaced. However, subjects who withdraw or are withdrawn from the study after dosing for reasons other than safety and tolerability, may be replaced after consultation between the SRC members. The total number of subjects dosed (including potential replacement subjects) will remain within a maximum of 10 subjects per cohort.

Subjects who withdraw or are withdrawn will be asked to remain at the clinic until the Investigator or designee agrees that the subject is fine and can be discharged. As soon as subject withdrawal is confirmed, blood sampling will be stopped. A PK blood draw may be collected at the time of withdrawal if deemed required by the Investigator. Study exit procedures will be performed at the time of withdrawal from the study or as soon as possible thereafter.

11.14 Adverse Events

11.14.1 Definition of Adverse Event

An AE is defined as any untoward medical occurrence in a patient or clinical trial subject administered a pharmaceutical product and which does not necessarily have a causal relationship with the treatment. An AE can, therefore, be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product.

11.14.2 Recording of Adverse Events

AEs will be recorded and evaluated for their seriousness, severity, and relationship to the study medication. AEs will be collected and documented from signing of ICF and throughout the course of the study. For a period of 60 days following the study treatment administration, AEs will be also documented, if reported. AEs will be followed-up until complete resolution, or until the Investigator judges it to be safe to discontinue follow-up. The relationship to the study treatment will be classified according to the clinical site SOPs.

11.14.3 Assessment of Severity

The severity of AEs will be graded according to criteria from the CTCAE (current version 5.0).

The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline (Table 14):

Table 14. Common Terminology Criteria for Adverse Events

GRADE	DESCRIPTION
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (instrumental activities of daily living refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.).
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living (self-care activities of daily living refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.

11.14.4 Assessment of Relationship to Study Drug

Each AE must be classified based on medical judgment and according to the following relationship categories: probable, possible, remote/unlikely, and unrelated. The definitions for the relationship categories are as follows (Table 15):

Table 15. Description of Adverse Event Relationship Categories

Category	Description
Probable	This category applies to AEs that are considered, with a high degree of certainty, to be related to the investigational product. An AE may be considered probable, if: 1. It follows a reasonable temporal sequence from the administration of the drug. 2. It cannot be reasonably explained by the known characteristics of the participant's clinical state, environmental or toxic factors or other modes of therapy administered to the participant. 3. It disappears or decreases on cessation or reduction in dose (there are important exceptions when an AE does not disappear upon discontinuation of the drug, yet drug relatedness clearly exists; e.g. (1) bone marrow depression, (2) tardive dyskinesias). 4. It follows a known pattern of response to the suspected drug. 5. It reappears upon re-challenge. [Related] Possible (must have first two points)
Possible	This category applies to AEs in which the connection with the investigational product administration appears unlikely but cannot be ruled out with certainty. An AE may be considered possible if, or when: 1. It follows a reasonable temporal sequence from the administration of the drug. 2. It may have been produced by the participant's clinical state, environmental or toxic factors, or other modes of therapy administered to the participant. 3. It follows a known pattern of response to the suspected drug.

Category	Description
Remote/Unlikely	In general, this category is applicable to an AE that meets the following criteria: 1. It does not follow a reasonable temporal sequence from the administration of the investigational product. 2. It may readily have been produced by the participant's clinical state, environmental or toxic factors, or other modes of therapy administered to the participant. 3. It does not follow a known pattern of response to the suspected drug. 4. It does not reappear or worsen when the investigational is re-administered.
Unrelated	This category is applicable to AEs that are judged to be clearly and incontrovertibly due only to extraneous causes (disease, environment, etc.), and do not meet the criteria for medication relationship listed under remote, possible, or probable.

11.14.5 Definition of Serious Adverse Event

A SAE is defined as any untoward medical occurrence that, at any dose:

- Results in death;
- Is life-threatening;
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect, or;
- Is otherwise considered to be an important medical event. The event may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above. Medical and scientific judgement should be exercised in deciding whether an event should be considered as an Important Medical Event. Examples of such medical events include:
  - Allergic bronchospasm requiring intensive treatment in an emergency room or at home;
  - Blood dyscrasias or convulsions that do not result in hospitalization;
  - Development of drug dependency or drug abuse.

Definitions of terms:

Life-threatening: An AE that is considered to be life-threatening when a subject is at immediate risk of death from the event as it occurred; i.e., it does not include a reaction that if it had occurred in a more serious form might have caused death.

Hospitalization: AEs requiring hospitalization should be considered SAEs. In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. When in doubt as to whether 'hospitalization' occurred or was necessary, the AE should be considered serious.

Hospitalization for elective surgery or routine clinical procedures that are not the result of AE (e.g., elective surgery for a pre-existing condition that has not worsened) need not be considered AEs or SAEs. If anything untoward is reported during the procedure, that occurrence must be reported as an AE, either 'serious' or 'non-serious' according to the usual criteria.

Disability/incapacity: An AE is incapacitating or disabling if the experience results in a substantial and/or permanent disruption of the subject's ability to carry out normal life functions.

11.14.6 Reporting of Serious Adverse Events

Any SAE will be reported to the Syneos Pharmacovigilance group via e-mail or fax within 24 hours of knowledge by the Investigator, and then in writing within 24 hours of awareness of the SAE. The notification must be directed to:

safetyreporting@syneoshealth.com
Fax: +1 877 464 7787

The Syneos Pharmacovigilance group will provide the report to the Sponsor. The notification must be directed to:

safetyreporting@neuracles.com

11.14.7 Suspected, Unexpected, Serious Adverse Drug Reactions

11.14.7.1 Fatal or Life-threatening Serious, Unexpected Adverse Drug Reactions

The Sponsor is responsible for notifying regulatory agencies of fatal or life-threatening serious, suspected, unexpected adverse drug reactions (by telephone, facsimile transmission or in writing) as soon as possible, but no later than 7 calendar days after becoming aware of the information. Additionally, within 8 days after having informed the agency(ies), a complete report must be submitted, including an assessment of the importance and implication of any findings. Syneos Health will handle notifications to the Canadian regulatory agency on behalf of the Sponsor.

It is the responsibility of the clinical site to report as soon as possible, but no later than 7 calendar days after first knowledge by the Investigator, fatal or life-threatening serious, suspected, unexpected adverse drug reactions to the IRB responsible for the study.

11.14.7.2 Other Suspected, Unexpected, Serious Adverse Drug Reactions

The Sponsor is responsible for notifying regulatory agencies of all other suspected, unexpected, serious adverse drug reactions that are neither fatal nor life-threatening as soon as possible, but no later than 15 calendar days after becoming aware of the information. Syneos Health will handle notifications to the Canadian regulatory agency on behalf of the Sponsor.

It is the responsibility of the clinical site to report to the IRB responsible for the study all other suspected, unexpected, serious adverse drug reactions that are neither fatal nor life-threatening, as soon as possible, but no later than 15 calendar days after first knowledge by the Investigator.

11.15 Pregnancy

In the event the female partner of a dosed male subject becomes pregnant during or shortly after participation in the study, this pregnancy will be reported to the Sponsor within 24 hours of first knowledge of the event. Follow-up information regarding the course and outcome of the pregnancy will be documented (after obtaining the consent of the female partner) as per site's SOP. If the outcome of the pregnancy meets the criteria for classification as an SAE, reporting of the event to the IRB and regulatory agency(ies) will be performed as per site's SOP.

11.16 Reportable Disease

In the case a subject has or manifests any clinical signs characteristic of a reportable disease or condition (e.g., HIV, tuberculosis, SARS, SARS-CoV-2), it is the responsibility of the Medical Director to notify the Public Health authorities within 48 hours after becoming aware of the information.

11.17 Premature Termination of the Study

The study may be prematurely terminated by the Investigator following consultation with the Sponsor, by the Sponsor or by the regulatory authorities. Following a decision to discontinue the trial, the Investigator will promptly inform the active study subjects and the IRB responsible for this trial, stating the reasons for discontinuation of the study and, furthermore, advise them in writing of any potential risks to the health of study subjects or other persons. It is the Sponsor's responsibility to report the premature termination of the study to the regulatory authority(ies), when required by the applicable regulatory requirement(s). The Canadian regulatory agency must be informed of premature termination within 15 days, provided with the reasons for the trial discontinuation and of any potential risks to the health of study subjects or other persons. Syneos Health may notify the Canadian regulatory agency on behalf of the Sponsor upon his request.

12. Analytical Methodology

When applicable, samples will be transported to the bioanalytical facility in at least two separate shipments, with each set of aliquots in separate shipments. Once the bioanalytical laboratory confirms receipt of the first shipment, the second set of aliquots may be sent. The samples should be packed on sufficient dry ice to keep them frozen for at least 72 hours.

The Bioanalytical Division of Syneos Health will analyze NS101 in serum and in CSF, FAM19A5 in CSF and in plasma, and ADAs/NAbs samples using validated methods. NAbs will be analyzed only for subjects with ADA positive results.

For PK, the primary sample will be sent to Syneos Health for bioanalysis. Back up sample shipment will be determined whether they will be shipped along with primary sample of next cohort. Detailed frequency of sample shipment will be described in Laboratory Manual for PK, ADAs, and NAbs.

Biomarker sampling for PD analysis (FAM19A5) will be done and analytical method will be technically transferred by Sponsor to Syneos Health's bioanalytical laboratory, so that sample storage condition and period of storage will need to be documented at that moment. Biobanking of samples for PD analysis (FAM19A5 ELISA) will be done after their collection. Samples will be stored under -70°C until analysis and will be analyzed only after completion of each cohort. Details on sample processing and storage will be included in a Laboratory Manual generated by Syneos Health's bioanalytical laboratory. Although there will be a time gap between collection and time of analysis, these biobanked samples will be analyzed only for PD biomarkers, as described above. No genetic analysis will be done.

The bioanalytical work in support of the study will be conducted in compliance with the GCP, using the SOPs in place in the Bioanalytical Division of Syneos Health. These SOPs are in accordance with applicable regulations in the industry: Guidelines on Bioanalytical Method Validation, Good Laboratory Practice (GLP), and Guideline for GCP ICH E6 (R2).

13. Safety/Tolerability, Pharmacokinetic and Statistical Analyses

PK analysis will be performed using Phoenix® WinNonlin®. Inferential statistical analyses will be performed using SAS® according to FDA guidelines.

13.1 Analysis Populations

13.1.1 Safety Population

The safety population is defined as all subjects who received any amount of study treatment and will be based on the actual treatment received.

13.1.2 Pharmacokinetic Population

The PK population will comprise all subjects who received any amount of NS101 and have sufficient post-dose serum concentration-time data to determine at least one PK parameter.

13.1.3 Pharmacodynamic Population

The PD population will include all subjects having received at least one dose of NS101, as appropriate, and for whom at least one post-dose PD parameter (FAM19A5 in plasma or CSF) can be adequately characterized.

13.1.4 Immunogenicity Population

The Immunogenicity population will include all subjects who have received any amount of NS101 and have at least one post-dose ADA/NAb measurement.

13.2 Safety and Tolerability Parameters and Analyses

Demographic parameters will be summarized descriptively.

Safety and tolerability to NS101 will be evaluated through the assessment of AEs (i.e., seriousness, severity, relationship to the study drug, outcome, duration, and management), vital signs, 12-lead ECG, clinical laboratory parameters, weight, and physical examination. TEAEs will be tabulated by study treatment. Changes from baseline values in vital signs, ECG, and clinical laboratory parameters tabulated by study treatment will be evaluated.

Safety and tolerability data will be reported using descriptive statistics. No inferential statistical analysis of safety data is planned. A complete description of the statistical analyses to be performed on the safety and tolerability data will be presented in the SAP.

13.3 Pharmacokinetics

13.3.1 Pharmacokinetics from Serum Samples

The following PK parameters will be calculated by standard non-compartmental methods for serum NS101:

1) $AUC_{0\text{-}24}$: area under the concentration-time curve from time zero to time 24 hours
2) $AUC_{0\text{-}t}$: Area under the concentration-time curve from time zero until the last observed concentration
3) $AUC_{0\text{-}inf}$: Area under the concentration-time curve from time zero to infinity (extrapolated)
4) Residual area: Percentage of $AUC_{0\text{-}inf}$ due to extrapolation from the time of the last observed concentration to infinity, calculated as $[1 – (AUC_{0\text{-}t}/AUC_{0\text{-}inf})] \times 100$
5) C_{max}: Maximal observed concentration
6) T_{max}: Time of C_{max}
7) $T_{½\ el}$: Terminal elimination half-life
8) K_{el}: Elimination rate constant
9) CL: total body clearance calculated as $Dose/AUC_{0\text{-}inf}$
10) V_z: apparent volume of distribution, calculated as $Dose / (K_{el} \times AUC_{0\text{-}inf})$

13.3.2 Pharmacokinetics from CSF Samples

A listing of all NS101 concentrations in CSF will be presented, as well as ratio of NS101 in serum to NS101 in CSF.

Additional PK analysis may be performed.

Upon Sponsor's request, PK repeats might be performed according to Syneos Health's SOP. If re-assays are requested for PK reasons, final results will include re-assay values, while results with original values will be presented in an appendix of the clinical study report as supportive data.

13.4 Pharmacodynamics

13.4.1 Pharmacodynamics from Plasma Samples

PD will be evaluated through the variation of level of FAM19A5 following administration of NS101. Baseline is defined as the Day 1 pre-dose PD sample. Changes in baseline FAM19A5 levels (Δ FAM19A5) will be determined. At each post-dose measurement of FAM19A5, the Day 1 pre-dose PD sample will be subtracted to calculate Δ FAM19A5.

The following PD parameters will be calculated for plasma FAM19A5:

1) $AUEC_{0-t}$: area under the PD effect versus time curve from time zero until the last measurable timepoint
2) E_{max}: maximal observed effect
3) TE_{max}: time of E_{max}

13.4.2 Pharmacodynamics from CSF Samples

A listing of all FAM19A5 concentrations in CSF will be presented.

13.5 Immunogenicity

The following will be assessed:

- Serum ADA
- NAbs

Incidence of ADAs and Nabs will be assessed. NAbs will be analyzed only for subjects with ADA positive results.

Immunogenicity results (the number of subjects with positive screening assay, confirmatory assay and titer assay) will be summarized by frequency tabulations. These results will be summarized by time point and dose level, which will include separate tables, and graphical frequency displays. The main PK parameters will be summarized by dose level and category of ADA status (at least one positive/negative).

Boxplots will present PK levels for each dose level by ADA status.

The details of the immunogenicity analysis will be outlined in greater detail in the SAP.

13.6 Statistical Analyses

Individual and mean serum or plasma concentration versus time curves will be presented for both linear and semi-log scales for NS101 and FAM19A5. Descriptive statistics (arithmetic and

geometric means, SD, CV%, Min, Max, and median) of the serum or plasma concentrations versus time will be presented as well for the PK and PD parameters.

Interim PK analyses will be performed for each cohort. Summary statistics will be used to describe the PK profile for this cohort. Dose proportionality may be assessed within different dose ranges if deemed appropriate with at least three doses using the power model approach. Dose-response relationship may be performed using Logistic Regression plots to assess the relationship between PK data after single dose administrations and specific AEs, when data permitting.

Summary statistics will be used to describe the PK profile for each cohort. After data base lock, the power model approach will be performed on the ln-transformed PK parameters AUC_{0-24}, AUC_{0-inf}, AUC_{0-t}, and C_{max}, and data to investigate dose-proportionality. Non-parametric analysis will also be performed on the untransformed T_{max} data.

14. Regulatory Considerations and Quality Assurance

14.1 Institutional Review Board Approval of Protocol and Other Study Documents

The Investigator(s) agree to provide the IRB with all appropriate documents, including a copy of the protocol/amendments, ICFs, advertising text (if any), Investigator's Brochure (if any) and any other written information provided to study subjects. The trial will not begin until the Investigators have obtained the IRB favourable written approvals for the above-mentioned study documents. A properly executed written ICF shall be read, signed, and dated by each subject prior to entering the trial or prior to performing any study procedure. The original signed and dated ICF will be kept at Syneos Health and a copy will be given to the subject.

In the event that the protocol is amended, the revised protocol must be approved by the IRB prior to its implementation, unless the changes involve only logistical or administrative aspects of the trial. If a revised ICF is introduced during the study, each subject's further consent must be obtained. The new version of the ICF must be approved by the IRB, prior to subsequently obtaining each subject's consent.

The Investigator and the Sponsor's representative must sign the protocol and its amendments (if any) before initiating the study.

It is the Sponsor's responsibility to submit the protocol and its amendments (if any), and the ICFs to regulatory authorities when necessary.

14.2 Compliance

This study will be conducted in compliance with the protocol, GCP, and all applicable regulations, including the Federal Food, Drug and Cosmetic Act, U.S. applicable Code of Federal Regulations (title 21), and any IRB requirements relative to clinical studies. The study will also be conducted in compliance with the recommendations laid down in the most recent version of the Declaration of Helsinki, with the exception that registration of such Phase 1 trials in a publicly accessible database is not mandatory. As required by the Canadian regulatory agency, a Clinical Trial

Application (CTA) will be submitted before the beginning of the study and a No Objection Letter (NOL) must be received prior to dosing.

14.3 Quality Assurance Program

Syneos Health has established Quality Control (QC) and Quality Assurance (QA) systems with written SOPs to ensure that the study will be conducted and data will be generated, recorded, and reported in compliance with the protocol, GCP, and applicable regulatory requirements. A rigorous QC program is applied to ensure accuracy of all data and reports. QA oversees a complementary risk-based program of audits to assure compliance with applicable regulations and Syneos Health's prescriptive documentation.

14.4 Audits, Inspections and Monitoring

In accordance with the principles of GCP and GLP, the study may be inspected by regulatory authorities, the Sponsor and Syneos Health. The Sponsor is entitled to access information about the status of the study and to review the original documents of the study.

15. Confidentiality and Retention of Study Records

This document contains trade secrets and commercial information that is confidential and may not be disclosed to third parties. Persons to whom this study protocol is disclosed must be informed that all the information herein is confidential and may not be further divulged. These restrictions will apply as well to all future communications if deemed privileged or confidential. Publication of the study results may only be allowed with written permission from the Sponsor.

All information on a subject obtained during the conduct of the study will be kept confidential. Subjects will be identified by an anonymized identifier on all samples and study records provided to the Sponsor or designee. In compliance with ICH GCP, the Sponsor’s authorized representatives, monitor(s), auditor(s), IRB, and regulatory authority(ies) will be granted direct access to the subject’s original trial-related records for verification of clinical trial procedures and/or data, without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations. Consent from the subject for disclosure of such information will be obtained in writing in the ICF. In addition, should a subject require medical care or hospitalization during the course of the study, the clinical site may contact the treating physician with the subject’s consent, except that consent may not be requested if there is an emergency situation. If the results of the study are published, the subject’s identity will remain confidential.

The clinical site will maintain adequate study records for 25 years after completion or termination of study. After this period, the Sponsor will be contacted to determine whether the study records will be forwarded to the Sponsor, destroyed or kept at the clinical site or another facility for a longer period of time at the Sponsor's expense.

16. References

1 NS101, Investigator's Brochure. Neuracle Science Co., LTD., Version 2.1. Dated: 25-OCT-2021.

2 Center for Drug Evaluation and Research (CDER), FDA. Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. July 2005.

3 Committee for Medicinal Products for Human Use (CHMP), EMA. Guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials with investigational medicinal products. EMEA/CHMP/SWP/28367/07 Rev. 1. 20 July 2017.

4 Sheehan, D.V., et al. Comparative Validation of the S-STS, the ISST-Plus, and the C-SSRS for Assessing the Suicidal Thinking and Behavior FDA 2012 Suicidality Categories. *Innov Clin Neurosci,* 2014. 11(9-10):32-46.

5 Sheehan, D.V., J.M. Giddens, and I.S. Sheehan. Status Update on the Sheehan-Suicidality Tracking Scale (S-STS), 2014. *Innov Clin Neurosci,* 2014. 11(9-10):93-140.