Official Title: A PHASE 1/2, OPEN-LABEL, DOSE-ESCALATION, SAFETY AND TOLERABILITY STUDY OF NC318 IN SUBJECTS WITH ADVANCED OR METASTATIC SOLID TUMORS

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A PHASE 1/2, OPEN-LABEL, DOSE-ESCALATION, SAFETY AND TOLERABILITY STUDY OF NC318 IN SUBJECTS WITH ADVANCED OR METASTATIC SOLID TUMORS

Sponsor Protocol Number: NC318-01

IND Number: 137910

Investigational Product: NC318
Humanized, IgG_1 Monoclonal Antibody Against Siglec-15 (S15)

Phase of Study: 1/2

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PROTOCOL SYNOPSIS

TITLE

A Phase 1/2, Open-Label, Dose-Escalation, Safety and Tolerability Study of NC318 in Subjects with Advanced or Metastatic Solid Tumors

HYPOTHESES

NC318 will be adequately tolerated following administration in multiple ascending doses to subjects with refractory solid tumors and that such administration may result in clinical benefit.

OBJECTIVES

Primary Objectives

1) To evaluate the safety, tolerability, and dose-limiting toxicities (DLTs) of NC318
2) Define a maximum tolerated dose (MTD) or pharmacologically active dose (PAD) of NC318 in subjects with advanced or metastatic solid tumors

Secondary Objectives

1) To evaluate the pharmacokinetic (PK) profile of NC318
2) To assess preliminary efficacy of NC318 by assessing objective response rate (ORR), duration of response (DoR), and disease control rate (DCR) per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1
3) To evaluate expression of Siglec-15 in tumor tissue and immune cell infiltrates at baseline and following NC318 treatment, and correlate expression level with efficacy and changes in tumor infiltrating lymphocytes

Exploratory Objectives

1) To assess the immunogenicity of NC318
2) To explore biomarkers that may predict the pharmacologic activity of NC318
3) To characterize the effect of NC318 on immune markers such as cytokine and immune cell phenotypes
4) To assess soluble S15 level at baseline and changes after NC318 treatment, and correlation with efficacy.

STUDY ENDPOINTS

Primary Endpoints

1) Safety and tolerability will be assessed by monitoring frequency, duration, and severity of adverse events (AEs). Note: Toxicity grading per NCI CTCAE v5.0

Secondary Endpoints

1) The endpoints for assessment of PK of NC318 include individual NC318 concentrations in serum and PK parameters.
2) The endpoints for assessment of antitumor activity/efficacy include objective response (OR) and disease control (DC) based on RECIST v1.1, DoR, progression-free survival (PFS), and overall survival (OS) as per RECIST v1.1
3) Tumor biopsy analyses to evaluate Siglec-15 (S15) expression in tumor tissue and immune cell infiltrates at baseline and following treatment, and correlate expression levels with efficacy and changes in tumor infiltrating lymphocytes.

Exploratory Endpoints

1) Immunogenicity, defined as the occurrence of anti-drug antibodies (ADA) to NC318 will be determined.
2) Biomarker effects of NC318 in peripheral blood and tumor tissue will be assessed, including but not limited to the following:
    a. Whole blood immune cell population profiling/immune-phenotyping.
    b. Plasma markers of inflammation or immune modulation (cytokine levels). Soluble S15 level at baseline and changes after NC318 treatment, and correlation with efficacy.
    c. The expression of additional biomarkers may also be assessed.

STUDY DESIGN

This is an open-label, nonrandomized, Phase 1/2 study to determine the safety and tolerability, define the MTD or PAD, and to assess the preliminary efficacy of NC318 in subjects with advanced or metastatic solid tumors. Subjects will receive NC318 on Day 1 of each cycle. Phase 1 of the study will begin with 14-day cycles; however, alternate dose administration schedules may also be explored depending on PK, pharmacodynamic (PD), biomarker, safety results, and feedback from investigators.

The study will be conducted in 2 parts:

- **Phase 1 – Dose Escalation and Safety Expansion** will determine the PAD, defined as a dose that provides a maximal biologic effect, such as an increase in biomarkers of immune activation or reduction of markers associated with immunosuppression, and/or the MTD of NC318, including defining the optimal dose administration schedule and the maximum number of tolerated doses (MNTD). **Phase 1 enrollment of the study is complete.**
- **Phase 2 – Safety Run-In and Dose Expansion** will further evaluate the safety, tolerability, preliminary efficacy, and PK/PD activity of NC318. **The study is currently enrolling Phase 2.**

Study Phase and Cohorts

Phase 1a – Dose Escalation

In Phase 1a, subjects with advanced or metastatic solid tumors who progressed after treatment with therapies known to confer clinical benefit, are intolerant to treatment, or refuse standard treatment were enrolled. A 3 + 3 design with escalating dose levels were explored to determine the MTD of NC318 (Table S1).

Table S1: NC318 Dose Levels and Cohorts

Cohort	Dose of NC318
-1 (Starting dose)	8 mg
1	24 mg
2	80 mg
3	240 mg
4	400 mg
5	800 mg
6	1600 mg

The Phase 1a design is summarized below:

A minimum of 3 evaluable subjects will be enrolled in each cohort beginning with Cohort -1 (8 mg; starting dose). The first 3 evaluable subjects within a cohort will be observed for a DLT observation period of 28 days, before the next cohort begins enrollment. Only one subject will be dosed on the first day of dosing for each cohort (additional subjects can begin in ≥48 hours). The dose will be escalated if 0 of the first 3 evaluable subjects enrolled has a DLT. If 1 of the first 3 evaluable subjects enrolled has a DLT, then the cohort will be expanded to include 3 additional evaluable subjects, and if no DLT occurs in the additional 3 subjects, then the dose will be escalated. If a DLT occurs in one-third or more of the expanded cohort, then the MTD will be deemed to be exceeded and the previous dose level will be considered the MTD. Intermediate dose levels below the MTD may be explored pending safety review. If only 3 subjects were treated at the MTD or PAD, then a minimum of 3 additional evaluable subjects will be enrolled at this dose before it is administered in Phase 2 of the study.

If Cohort -1 (8 mg; starting dose) exceeds the MTD, the sponsor and investigators will consider dosing NC318 at a lower dose, and/or investigate 8 mg at alternate dose schedules, based on available safety, PK, PD, and biomarker data. If an alternate schedule is determined to be safe, re-escalation of NC318 will proceed according to the table below.

Throughout the treatment period, if > 33% of subjects (a minimum of 6 subjects) experience a ≥ Grade 3 toxicity related to study drug after completing ≥ 4 cycles, then dose administration will be stopped, and the MNTD will be determined in conjunction with the investigators and sponsor based on all available safety data.

Subjects who drop out for reasons other than a DLT (e.g., events clearly associated with the underlying disease, disease progression, concomitant medication, or comorbidity), during the 28-day DLT observation period will be considered non-evaluable and will be replaced. Dose modifications should not be made during the DLT observation period without discussion with the medical monitor. Intrasubject dose escalation is not permitted; however, once the recommended Phase 2 dose (RP2D) and schedule has been determined, ongoing subjects in Phase 1 may be permitted to escalate to the RP2D with approval of the medical monitor.

Phase 1b – Safety Expansion

The purpose of Phase 1b was to evaluate additional PD activity of NC318 and confirm the preliminary safety of the dose escalation cohorts from Phase 1a. Several cohorts from Phase 1a were expanded at doses and schedules equivalent to or lower than the highest dose levels determined to be safe and/or doses determined to be pharmacologically active.

The following cohorts were expanded during Phase 1b: Cohort 2 (80mg), Cohort 3 (240mg), and Cohort 4 (400mg). Phase 1 enrollment of the study is complete. A total of 49 subjects were enrolled across Phase 1a and Phase 1b.

The Phase 1b design is summarized below:

Approximately 36 evaluable subjects will be enrolled in the Phase 1b safety expansion, with each cohort enrolling approximately 9 evaluable subjects. If < 3 of 9 evaluable subjects experience a DLT, the cohort will be deemed safe. If > 1 safety expansion cohort is deemed safe, then a recommended dose and schedule will be determined in conjunction with the investigators and sponsor based on all available safety, PK, PD, and biomarker results. The safety expansion cohorts may be conducted in parallel to Phase 2 and may be limited by the sponsor to subjects with specific tumor types to achieve a balance across cohorts.

Phase 2

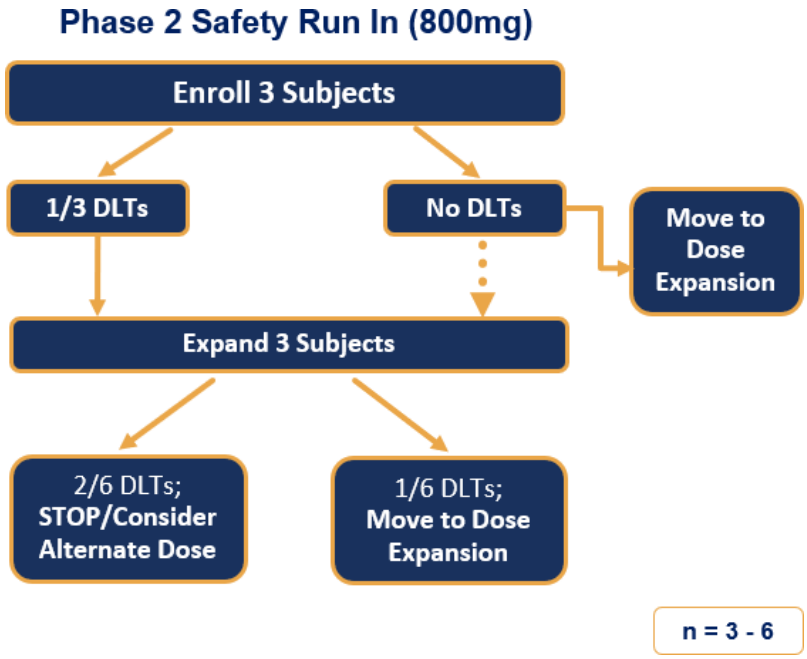
The trial is currently enrolling Phase 2. Phase 2 will evaluate subjects with advanced or metastatic solid tumors characterized as Tumor Mutational Burden High (TMB-H) and/or MicroSatellite Instability High (MSI-H)/ deficient MisMatch Repair (dMMR). The following tumor types will be enrolled: Lung, Head and Neck Squamous Cell Carcinoma (HNSCC, Breast, Endometrial, Melanoma, CRC, Urothelial, Cholangiocarcinoma, and other tumors known to be TMB-H and/or MSI-H/dMMR.

Previously as part of the initial Phase 2 design, a RP2D of 400mg Q2W was tested. 47 subjects were treated at the initial RP2D. Based on further ongoing evaluation of the PK/PD studies from Phase 1/2, the RP2D was adjusted to 800mg with an alternative administration schedule. To further evaluate the new RP2D, a Phase 2 Safety Run-In will be conducted utilizing a 3+3 design (Figure S1).

Phase 2 - Safety Run-in:

A minimum of 3 evaluable subjects will be enrolled. The first 3 evaluable subjects will be observed for a DLT observation period of 28 days. Only one subject will be dosed on the first day of dosing (additional subjects can begin in ≥ 48 hours). If there are no DLTs observed in the first 3 subjects, then Phase 2 Dose Expansion may begin at the new RP2D. If 1 of the first 3 evaluable subjects enrolled has a DLT, then the cohort will be expanded to include 3 additional evaluable subjects. If there are no DLTs in the additional 3 subjects, then Phase 2 Dose Expansion may begin at the new RP2D. If a DLT occurs in one third or more of the 3 additional subjects then the enrollment will be paused and intermediate dose levels or alternative dose schedules may be considered.

Figure S1: Phase 2 Safety Run-In Design



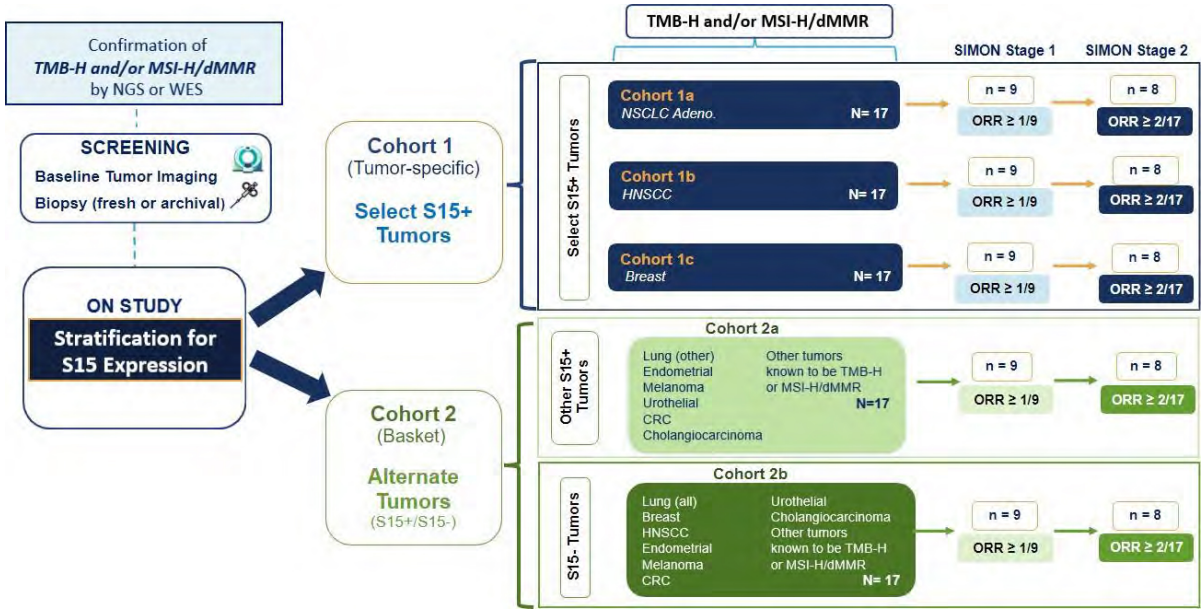
Abbreviation: DLT = dose-limiting toxicity

Phase 2 - Dose Expansion:

Phase 2 Dose Expansion will further evaluate the safety, tolerability, preliminary efficacy, and PK/PD activity of NC318 at the RP2D utilizing a Simon 2-stage design (Figure S2). Subjects will be stratified after enrollment into two main cohorts based on S15 expression and tumor type:

- Cohort 1: Select S15+ Tumors (Lung (adenocarcinoma), HNSCC, Breast)
- Cohort 2: Alternate Tumors (including Other S15+ Tumors and S15- Tumors)

Figure S2: Phase 2 Dose Expansion (Simon 2-Stage Design)



Abbreviations: CRC = colorectal cancer; dMMR = deficient DNA mismatch repair; MSI = microsatellite instability high; NGS = next generation sequencing; NSCLC = non-small cell lung cancer; ORR = objective response rate; TMB-H = Tumor mutational burden high; WES = whole exome sequencing.

Cohort 1: Select S15+ Tumors

This cohort will include subjects who are 1) confirmed as TMB-H and/or MSI-H/dMMR, 2) express S15, and 3) have one of the following tumor types, separated into tumor-specific subgroup:

- **Cohort 1a:** NSCLC (adenocarcinoma)
- **Cohort 1b:** HNSCC
- **Cohort 1c:** Breast cancer

Each subgroup will be independently assessed and guided by the Simon 2-stage design and will enroll 9 evaluable subjects in Stage 1. If no responses are observed within a subgroup, then the subgroup will be discontinued. If at least 1 response is observed, Stage 2 will begin and enroll 8 additional evaluable subjects, for a total of 17 evaluable subjects in each subgroup (1a, 1b, and 1c).

Cohort 2: Alternate Tumors (including Other S15+ Tumors and S15- Tumors)

This basket cohort will include all other enrolled subjects who are confirmed as TMB-H and/or MSI-H/dMMR but do not fall under the criteria to be included in Cohort 1. Based on results of S15 expression, subjects will be further stratified into two subgroups:

- **Cohort 2a:** Other S15+ Tumors
- **Cohort 2b:** S15- Tumors

Each subgroup will be independently assessed and guided by the Simon 2-stage design and will enroll 9 evaluable subjects in Stage 1. If no responses are observed within a subgroup, then the sub-group will be discontinued. If at least 1 response is observed, Stage 2 will begin and enroll 8 additional evaluable subjects, for a total of 17 evaluable subjects in each subgroup (2a and 2b).

DOSE SELECTION AND ADMINISTRATION SCHEDULE

During Phase 1 Dose Escalation, various dose levels were evaluated ranging from 8 mg to 1600 mg. During the initial Phase 2 design, a RP2D of 400 mg Q2W was tested. Based on further evaluation of the PK/PD studies from the original Phase 1/2 design, the RP2D has been adjusted to 800 mg with an alternative administration schedule.

The new RP2D and administration schedule will be as follows:

- **Initial Dosing:** 800 mg weekly for 8 Cycles
- **Subsequent Dosing (Cycle 9 onwards):** 800 mg every 2 weeks

Subjects will have regularly scheduled visits on Day 1 of every cycle. The duration of each cycle is dependent on the dosing schedule:

- Initial Dosing: 800 mg weekly for 8 Cycles
  - **Cycle Duration:** 7 days (+3 days after Cycle 1)

- Subsequent Dosing (Cycle 9 onwards): 800 mg every 2 weeks
  - **Cycle Duration:** 14 days (±3 days)

Subjects will begin with the Initial Dosing Schedule on Cycle 1 (Day 1) and continue with weekly dosing for 8 Cycles. Beginning with Cycle 9, subjects will follow the Subsequent Dosing Schedule of 800 mg every 2 weeks. **Note:** Cycle 9 should occur two weeks following the last weekly dose of NC318. Subjects will remain on the Subsequent Dosing Schedule of 800 mg every two weeks until disease progression, withdraw of consent, or intolerable toxicity (whichever comes first).

TARGET SUBJECT POPULATION

Men and women, 18 years or older, with advanced solid malignancies that can be evaluable by RECIST criteria, that progressed on standard treatment, who have been unable to tolerate standard therapy, who have refused standard therapy, or for whom no standard anticancer therapy exists may be enrolled. Subjects must provide written informed consent and have adequate organ function. Presence of measurable disease based on RECIST v1.1, and consent to have a non-target lesion biopsied before treatment. **Note:** Archival tissue may be submitted in place of fresh biopsy at pre-treatment. If a subject is scheduled to have a tumor biopsy for the purposes of this study and it is subsequently determined that tumor tissue cannot safely be obtained, then the subject may still enroll in the study. Subjects with certain serious medical conditions (in addition to the diagnosis of cancer) would be excluded from participation in the trial.

ELIGIBILITY CRITERIA

Inclusion Criteria

A subject who meets all the following criteria may be included in the study:

1) Men and women aged 18 or older.
2) Willing and able to provide written informed consent for the study.
3) ECOG performance status 0 to 1.
4) Subjects with advanced unresectable and/or metastatic solid tumors confirmed to be TMB-H and/or MSI-H/dMMR including: Lung, Breast, HNSCC, Endometrial, Melanoma, CRC, Urothelial, Cholangiocarcinoma, and other tumors known to be TMB-H and/or MSI-H/dMMR.
5) Subjects who have disease progression after treatment with available therapies that are known to confer clinical benefit, or who are intolerant to treatment, or who refuse standard treatment.

   Note: There is no limit to the number of prior treatment regimens.
6) Presence of measurable disease based on RECIST v1.1. Tumor lesions situated in a previously irradiated area, or in an area subjected to other locoregional therapy, are not considered measurable unless there has been demonstrated progression in the lesion.
7) Able to provide pretreatment tumor tissue sample (archival ≤ 5 years old) or undergo tumor biopsy at Screening (must allow for adequate sample of tissue from appropriate site).

   Note: Formalin-fixed, paraffin embedded (FFPE) tissue blocks are preferred to slides. Newly obtained biopsies are preferred to archived tissue.
8) Subjects of childbearing potential (defined as female subjects who have not undergone surgical sterilization with a hysterectomy and/or bilateral oophorectomy and are not postmenopausal, defined as ≥ 12 months of amenorrhea not caused by reversible conditions, diseases, or medications) and non-sterilized male subjects must agree to take appropriate precautions to avoid pregnancy or fathering children (with at least 99% certainty) from screening through 90 days after the last dose of study drug.

   Note: Female subjects of childbearing potential must have negative serum pregnancy test at screening.

Subject Exclusion Criteria

A subject who meets any of the following criteria will be excluded from the study:

1) Inability to comprehend or unwilling to provide informed consent.
2) Laboratory and medical history parameters not within the Protocol-defined range.
   a. Absolute neutrophil count < 1.5×10^9/L.
   b. Platelets < 100×10^9/L.
   c. Hemoglobin < 9 g/dL or < 5.6 mmol/L.
   d. Serum creatinine > 1.5 × institutional upper limit of normal (ULN) OR measured or calculated creatinine clearance < 30 ml/min for subjects with creatinine levels <1.5 × institutional ULN.
   e. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) > 2.5 × ULN. **Note**: Subjects with liver metastases are permitted to enroll if AST/ALT is ≤ 5 × ULN.
   f. Total bilirubin > 1.5 × ULN. **Note**: Subjects with documented Gilbert's syndrome with elevated baseline total bilirubin ≤ 3.0 mg/dL may be enrolled.
   g. International normalized ratio (INR)/prothrombin time (PT) > 1.5 × ULN or activated partial thromboplastin time (aPTT) > 1.5 × ULN; applies only to subjects who do not receive therapeutic anticoagulation; subjects receiving therapeutic anticoagulation should be on a stable dose.

3) Transfusion of blood products (including platelets or red blood cells) or administration of colony-stimulating factors (including granulocyte colony-stimulating factor, granulocyte macrophage colony–stimulating factor, or recombinant erythropoietin) within 7 days prior to first administration of study drug.
4) Receipt of anticancer medications or investigational drugs within the following intervals before the first administration of study drug:
    a. ≤ 14 days for chemotherapy, targeted small molecule therapy, or radiation therapy. Subjects must not have had radiation pneumonitis because of a treatment. A 1-week washout is permitted for palliative radiation to non–central nervous system (CNS) disease provided recovery is adequate.

       Note: Bisphosphonates and denosumab are permitted medications.
    b. ≤ 28 days for prior immunotherapy or persistence of active cellular therapy (e.g., chimeric antigen receptor T cell therapy; other cellular therapies must be discussed with the medical monitor to determine eligibility).
    c. ≤ 28 days for a prior mAb used for anticancer therapy except for denosumab.
    d. ≤ 7 days for immune-suppressive–based treatment for any reason.

       Note: Use of inhaled or topical steroids or corticosteroid use for radiographic procedures is permitted.

       Note: The use of corticosteroids equivalent to prednisone ≤ 10mg/day is allowed.
    e. ≤ 28 days or 5 half-lives (whichever is longer) before the first dose for all other investigational study drugs or devices.
    f. ≤ 14 days for a COVID-19 vaccine.

       Note: For 2-dose COVID-19 vaccines, subjects must wait at least 14-days after 2nd dose administration.
5) Has not recovered to ≤ Grade 1 from toxic effects of prior therapy (including prior immunotherapy) and/or complications from prior surgical intervention before starting therapy.

   Note: Subjects with stable chronic conditions (≤ Grade 2) not expected to resolve (such as neuropathy and alopecia) are exceptions and may enroll.

   Note: Subjects with a history of any grade immune-related ocular AE (e.g., episcleritis, scleritis, uveitis) will be excluded.

   Note: Subjects with a history of a Grade 3 or higher irAE from prior immunotherapies are excluded from the Phase 1a dose-escalation portion of the study.
6) Receipt of a live vaccine within 30 days of planned start of study therapy.

   Note: Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox/zoster, yellow fever, rabies, Bacillus Calmette–Guérin, and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines and are not allowed.
7) Active autoimmune disease that required systemic treatment in the past (i.e., with use of disease-modifying agents, corticosteroids, or immunosuppressive drugs).

   Note: Subjects with hyperthyroidism or hypothyroidism, who have not required any systemic treatment for an autoimmune disease for at least 2 years and have normal thyroid function or are stable on thyroid hormone replacement are allowed to participate in the study.

Note: Replacement and symptomatic therapies (e.g., levothyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) are not considered a form of systemic immune suppressive therapy and are allowed.

8) Known active CNS metastases and/or carcinomatous meningitis.

   Note: Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least 28 days before the first dose of study drug and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases or CNS edema, and have not required steroids for at least 7 days before the first dose of study drug.

9) Known additional malignancy that is progressing or requires active treatment, or history of other malignancy within 2 years of study entry after treatment with curative intent.

   Note: Cured basal cell or squamous cell carcinoma of the skin, superficial bladder cancer, prostate intraepithelial neoplasm, carcinoma in situ of the cervix, or other noninvasive or indolent malignancy, or cancers from which the subject has been disease free for > 1 year are not considered exclusionary.

10) Has a history of (non-infectious) pneumonitis / interstitial lung disease that required steroids or has current pneumonitis / interstitial lung disease.

11) Documented known activating or driver mutations (i.e. EGFR mutations/amplification, BRAF mutations, ALK alterations, etc.) which have not been previously treated with a standard of care targeted therapy.

12) Subjects with screening QTc interval > 470 milliseconds (corrected by Fridericia) are excluded.

13) Uncontrolled systemic fungal, bacterial, viral, or other infection despite appropriate anti-infection treatment.

14) Evidence of hepatitis B virus (HBV) or hepatitis C virus (HCV), unless the hepatitis is considered to be cured.

   Note: Subjects with no prior history of hepatitis B infection who have been vaccinated against hepatitis B and who have a positive antibody against hepatitis B surface antigen test as the only evidence of prior exposure may participate in the study.

   Note: If HCV/HBV antibody or antigen tests are positive, reflex testing (HCV RNA and/or HBV DNA tests) should be performed prior to assessing subject eligibility.

   Note: Subjects with prior hepatitis B virus [HBV] infection must have HBV viral load [VL] < 100 IU/mL before study enrollment and must be treated according to local standards; hepatitis C virus [HCV] infection must have, before study enrollment, no detectable VL and must be treated according to local standards.

15) Known history of HIV (HIV 1 or HIV 2 antibodies).

16) Known allergy or reaction to any component of study drug or formulation components.

17) Is pregnant or breastfeeding or expecting to conceive or father children within the projected duration of the study, starting with the screening visit through 90 days after the last dose of study treatment.

18) Any condition that would, in the investigator's judgment, interfere with full participation in the study, including administration of study drug and attending required study visits; pose a significant risk to the subject; or interfere with interpretation of study data.

INVESTIGATIONAL PRODUCT, DOSAGE, AND MODE OF ADMINISTRATION

NC318 will be administered by intravenous (IV) infusion, over a minimum of 30 minutes on Day 1 of each cycle. Subjects will continue to receive NC318 until disease progression, withdraw of consent, or intolerable toxicity (whichever comes first). After completing 12 months of treatment, if the subject is deriving benefit from NC318 and has not met any of the protocol defined conditions for withdrawal or discontinuation, the subject will be followed by the PI per standard of care. Subjects approved to continue treatment will remain on dose level assigned at the time of enrollment (unless otherwise approved for dose modification by the Sponsor). Safety reporting, documentation of disease assessments, and collection of survival status will continue per protocol.

STATISTICAL ANALYSIS PLAN

The sample size for this study will be determined so that sufficient subjects are included to assess the safety, tolerability, PK, and PD of repeat doses of NC318. The sample size is not fixed and will vary based on emerging safety data at the doses studied.

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LIST OF ABBREVIATIONS

Abbreviation or Specialized Term	Definition
ADA	Anti-Drug Antibody
ADCC	Antibody Dependent Cellular Cytotoxicity
AE	Adverse Event
ALT	Alanine Aminotransferase
ALK	Anaplastic lymphoma kinase
aPTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
AUC	Area Under the Curve
BRAF	v-Raf murine sarcoma viral oncogene homologue B1
BSA	Body Surface Area
CDC	Complement Dependent Cytotoxicity
CI	Confidence Interval
CL	Clearance
C_{max}	Maximum Concentration of Drug
CNS	Central Nervous System
CR	Complete Response
CRC	Colorectal Cancer
CRF	Case Report Form
CRS	Cytokine Release Syndrome
CT	Computed Tomography
CTLA-4	Cytotoxic T-Lymphocyte–Associated Protein 4
DC	Disease Control
DCR	Disease Control Rate
DLT	Dose-Limiting Toxicity
dMMR	Deficient DNA Mismatch Repair
DNA	Deoxyribonucleic Acid
DoR	Duration of Response
EC_{30}	Effective Concentration for 30% of Maximum Effect
EC_{50}	Effective Concentration for 50% of Maximum Effect
ECG	Electrocardiogram
ECL	Electrochemiluminescence
ECOG	Eastern Cooperative Oncology Group

Abbreviation or Specialized Term	Definition
eCRF	Electronic Case Report Form
ED_{30}	Effective Dose for 30% of Maximum Effect
EGFR	Epidermal growth factor receptor
EOT	End-of-Treatment
FAAN	Food and Allergy Anaphylaxis Network
FAS	Full Analysis Set
FIH	First-in-Human
FFPE	Formalin-Fixed Paraffin-Embedded
FoxP3	Forkhead Box P3
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HED	Human-Equivalent Dose
HIV	Human Immunodeficiency Virus
HNSCC	Head and Neck Squamous Cell Carcinoma
IB	Investigator's Brochure
IBW	Ideal Body Weight
ICF	Informed Consent Form
ICH	International Council for Harmonization
IEC	Independent Ethics Committee
Ig	Immunoglobulin
IND	Investigational New Drug
INR	International Normalized Ratio
INs	Investigator Notifications
IP	Intraperitoneal
irAE	Immune-Related Adverse Event
IRB	Institutional Review Board
IV	Intravenous
KO	Knock Out
LKA	Last Known Alive
LPS	Lipopolysaccharide

Abbreviation or Specialized Term	Definition
mAb	Monoclonal Antibody
MedDRA	Medical Dictionary for Regulatory Activities
MNTD	Maximum Number of Tolerated Doses
mRECIST	Modified Response Evaluation Criteria in Solid Tumors
MRI	Magnetic Resonance Imaging
MSI-H	Microsatellite Instability-High
MTD	Maximum Tolerated Dose
NAb	Neutralizing Antibody
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NIAID	National Institute of Allergy and Infectious Disease
NOAEL	No Observed Adverse Effect Level
NSAID	Nonsteroidal Anti-Inflammatory Drug
NSCLC	Non-Small Cell Lung Cancer
ORR	Objective Response Rate
OR	Objective Response
OS	Overall Survival
PAD	Pharmacologically Active Dose
PAS	PK Analysis Set
PBMC	Peripheral Blood Mononuclear Cells
PD	Pharmacodynamics
PD-1	Programmed Cell Death Protein 1
PD-L1	Programmed Cell Death Protein Ligand 1
PEF	Peak Expiratory Flow
PFS	Progression-Free Survival
PI	Principal Investigator
PK	Pharmacokinetics
PR	Partial Response
PT	Prothrombin Time
QTcF	Fridericia Correction
RCC	Renal Cell Carcinoma
RECIST	Response Evaluation Criteria in Solid Tumors
RNA	Ribonucleic Acid
RP2D	Recommended Phase 2 Dose

Abbreviation or Specialized Term	Definition
S15	Siglec-15
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Safety Analysis Set
SC	Subcutaneous
SD	Study Day
Siglec	Sialic acid-binding Immunoglobulin Lectins
SSD	Safe Starting Dose
SUSAR	Suspected Unexpected Serious Adverse Reaction
$t_{½}$	Half-Life
TEAE	Treatment-Emergent Adverse Event
TIL	Tumor Infiltrating Lymphocyte
TK	Toxicokinetic
TMB	Tumor mutational burden
TME	Tumor Microenvironment
TNBC	Triple Negative Breast Cancer
TPS	Tumor Proportion Score
Treg	Regulatory T cell
ULN	Upper Limit of Normal
US FDA	United States Food and Drug Administration
USA	United States of America
V_d	Volume of Distribution
WBDC	Web Based Data Capture

1. INTRODUCTION

This is a Phase 1/2, multicenter, open-label, dose-escalation study. The study will be conducted in 2 parts. Phase 1 will utilize a 3 + 3 design to determine the maximum tolerated dose (MTD) or pharmacologically active dose (PAD) for NC318 in subjects with advanced or metastatic solid tumors. Phase 2 of the study will further evaluate the safety, tolerability, preliminary efficacy, pharmacokinetics (PK), and pharmacodynamics (PD) of the recommended Phase 2 dose (RP2D) of NC318. **The study is currently enrolling Phase 2.**

1.1. Background

1.1.1. The Role of the Immune System in Cancer

The immune system is comprised of diverse sets of cells designed to protect a host from pathogens while distinguishing from host and foreign antigens. This immune response is controlled by a series of checks and balances to allow for robust immune responses to pathogens while preventing either an excessive inflammatory event or an autoimmune response. Through immune surveillance, the immune system has been shown to recognize, attack, and destroy tumor cells (Wolchok 2008). The presence of tumor infiltrating lymphocytes (TIL) in cancer tissue among various malignancies has been shown to confer a more favorable prognosis (Uppaluri 2008; Bellati 2009; Oble 2009; Nosho 2010; Shirabe 2010; Bremnes 2011; Gooden 2011; Schreiber 2011; Talmadge 2011; Mei 2014; Salgado 2015). Detailed analysis of $CD8^+$ T cells and the ratio of $CD8^+$ effector T cells/forkhead box P3 $(FoxP3)^+$ regulatory T cells (Tregs) seem to correlate with improved prognosis and long-term survival in many solid tumors (Salgado 2015). Although the immune system has been shown to recognize and reject a tumor, many tumors evade immune surveillance or develop mechanisms of resistance.

Histologic evaluation of multiple human cancers shows extensive infiltration by pro-inflammatory immune cells (Galon 2006), that are immune-suppressive leading to tumor progression. In some cases, as tumors grow, an "equilibrium" may be reached where tumor growth is matched by immune-mediated tumor destruction. However, malignant cells may accumulate certain mutations, making them nonimmunogenic, and/or immunosuppressive pathways become activated, allowing the tumor to escape immune recognition (Dunn 2002; Schreiber 2011; DuPage 2012; Matsushita 2012). The accumulation of suppressive cells and an inhibitory cytokine milieu in and around a tumor can form an immunosuppressive environment that prevents successful T cell–mediated destruction of malignant cells (Schaer 2011). This balance of immune control is not limited to lymphocytes. For example, although M1 macrophages secrete pro-inflammatory cytokines that stimulate immunity (including anti-tumor immune responses), the M2 macrophages have been described as promotors of immunosuppression and tumor progression (Sica 2006). Overcoming tumor resistance to immune surveillance either through stimulating the immune response or preventing inhibition is the basic rationale for the development of immunotherapies.

Targeting the immune system is a proven and effective approach for cancer therapies. United States Food and Drug Administration (US FDA)–approved checkpoint inhibitors, such as ipilimumab, nivolumab, and pembrolizumab, allow for the immune response to continue to proliferate by preventing inhibitory signals. Preventing Siglec-15 (S15) mediated immune

suppression through M2 Macrophages and S15^{+} tumors is another promising treatment approach and is the focus of this clinical trial.

1.1.2. Immune Modulators

Immune cell receptors known as checkpoint modulators (collectively known as immune modulators) provide a critical mechanism for the regulation of an immune response. Checkpoint modulation can either diminish an immunosuppressive process or stimulate T cell responses to enhance the overall immune response and prevent or reverse tumor progression. Modulation of coinhibitory and costimulatory receptors of the immune system has become a proven approach for the immunotherapy of cancer (Chen 2013).

The development of fully human antibodies that target and modulate immunomodulatory proteins in humans have led to the discovery of multiple validated targets for the immunotherapy of cancer (Leach 1996; Chen 2013). Antibodies that engage the various checkpoint modulators can broadly be classified into two categories based on mechanism of action: antagonists (blocking the interaction between receptor and cognate ligand[s]), and agonists (inducing or facilitating receptor-mediated signaling pathways). Clinical testing of therapeutic antibodies has demonstrated their ability to influence the direction and magnitude of the immune responses, leading to tumor eradication (Yao 2013). The blocking of coinhibitory receptors such as cytotoxic T-lymphocyte–associated protein 4 (CTLA-4) or programmed cell death protein 1 (PD-1) blockade are the basis of US FDA approved therapies to augment an antitumor immune response. Preclinical research has demonstrated a rationale for targeting the inhibitory receptor S15 found on tumor cells and myeloid cells (Wang 2019).

1.1.3. Siglec-15 and Immunity

S15 is a member of the Siglec family (Sialic acid-binding Immunoglobulin Lectins), a distinct subgroup of immunoglobulin (Ig) superfamily proteins involved in the discrimination between self and non-self-immune regulation (Macauley 2014). It is a type-I transmembrane protein consisting of two Ig-like domains (IgV and IgC) and a short cytoplasmic tail. It has been implicated in playing a role in immune suppression.

Studies of S15 knock out (KO) mice led to mice developing without physical abnormalities other than slightly increased trabecular bone mass and mild osteopetrosis (Hiruma 2013). Like the findings by Hiruma et al., KO mice generated in the Chen lab did not exhibit any noticeable phenotype (Wang 2019). However, follow-on functional studies demonstrated that mice with S15 deficiency have enhanced pro-inflammatory cytokine production upon lipopolysaccharide (LPS) stimulation and enhanced antigen-specific T cell responses *in vivo*, suggesting a role for S15 in mediating immunosuppression. Subsequent studies demonstrated a key role for S15-positive (S15^{+}) myeloid cells in mediating immunosuppression. The S15 KO mice demonstrate that absence of S15^{+} expression results in a decrease in tumor progression and an increase in survival for the glioma GL261 and B16.GMCSF melanoma murine tumor models. Taken together these findings suggest a tumor immune-escape mechanism and opportunity to target S15 as a means of enhancing immune function and antitumor responses in the tumor microenvironment (TME).

The interaction of S15 with its receptor mediates immunosuppression. NC318 blocks the interactions between S15 and its receptor which is expressed on myeloid cells and the T cells of the TME thereby relieving inhibitory signaling and blocking immunosuppression. The rationale

for developing NC318 for cancer is based on nonclinical models that demonstrate that the targeting of S15 can improve the immune response and provide benefit in multiple oncology indications.

1.2. Overview of NC318

NC318 is a humanized modified $IgG_{1\kappa}$ monoclonal antibody (mAb) that binds to the extracellular domain of the human S15 receptor. Blocking S15 with NC318 may prevent immune suppression and result in augmented immune responses including some anti-tumor immune responses. NC318 is being developed for the treatment of advanced malignancies.

1.2.1. Pharmacokinetics of NC318: Pre-clinical Data

Single-dose and repeat-dose PK of NC318 were characterized in both Sprague Dawley rats and cynomolgus monkeys. Concentrations of NC318 in serum were analyzed using a quantitative electrochemiluminescence (ECL) method developed by Meso Scale Discovery (MSD, Rockville, MD). Immunogenicity testing was also conducted in conjunction with these studies. Serum samples were screened for the presence of anti-drug antibodies (ADA) and confirmed by immune-competition with free NC318.

Repeat-dose PK of NC318 was first investigated in a Good Laboratory Practices (GLP)-compliant repeat-dose study in Sprague Dawley rats. Animals received 3, 30, or 100 mg/kg NC318 (N=9/sex/group; 3/sex/timepoint) by IV tail vein injection on SD 1, 8, 15, 22 and 29. PK analysis (excluding ADA-positive animals) showed that exposure (C_{max}, and AUC) of NC318 was linearly proportional to dose level, across the range of doses that were tested. Accumulation of NC318 was observed with every week dosing. Mean volume of distribution ranged from 51.0 to 80.2 ml/kg on SD 1 and from 24.8 to 54 ml/kg on SD 29, indicating limited distribution outside of the vasculature. Mean CL on SD 29 ranged from 0.08 to 0.19 ml/h/kg. PK parameters were generally equivalent in both genders. The overall mean terminal $t_{½}$ was 6 to 10 days following the fifth dose.

Repeat-dose PK of NC318 was next examined in a GLP-compliant repeat-dose study in cynomolgus monkeys. Animals were administered with 0, 3, or 30 mg/kg NC318 (N=5/ sex/group; 3/sex Main Group and 2/sex Recovery Group) on SD 1, 8, 15, 22, and 29 by 30-minute IV infusion. Animals at the highest dose level received 100 mg/kg NC318 (N=5/sex) on SD 1 and 60 mg/kg/dose on SD 8, 15, 22, and 29. The dose level for the highest dose was lowered beginning with the second dose due to periorbital swelling observation in male animals and severe toxicity in 2 female monkeys that required early sacrifice following the 100 mg/kg dose on SD 1. ADA were observed in 6 of the NC318-treated animals, which was associated with accelerated CL after the fifth dose. PK analysis showed that exposure (C_{max} and AUC) of NC318 was linearly proportional to dose level, across the range of doses that were tested. Slight accumulations were observed with every week dosing. The mean volume of distribution (V_d) and mean CL) were generally comparable for males and females at all dose levels on SD 1 and SD 29. Mean V_d ranged from 60 to 80 ml/kg on SD 1 and from 38.8 to 117 ml/kg on SD 29. Mean CL on SD 29 ranged from 0.12 to 0.71 ml/h/kg. PK parameters were generally equivalent in both genders. The overall mean terminal $t_{½}$ was 5 - 9 days following the fifth dose.

The presence of ADA in dosed rats and cynomolgus monkeys correlated with more rapid CL. Immunogenicity of human protein therapeutics in non-human species is a common occurrence, and immunogenicity data from animals have little value for predicting the risk of immunogenicity in humans, as discussed in International Council for Harmonization (ICH)-S6(R1) and (Ponce

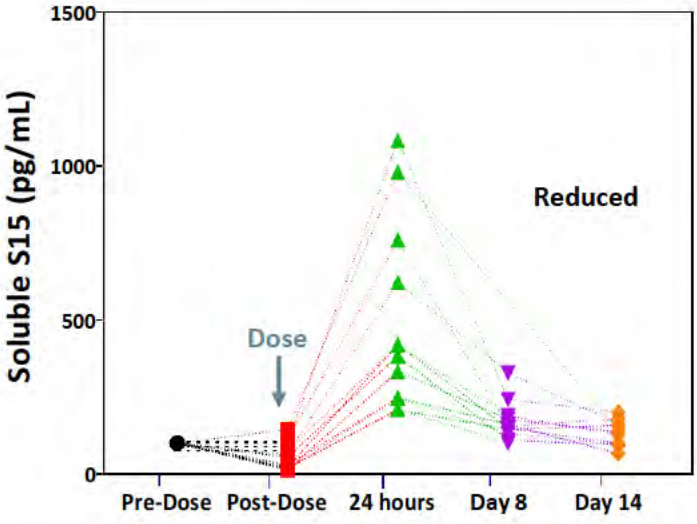
2009). Sufficient drug exposure and pharmacologic activity were maintained in both studies to allow for meaningful toxicological evaluations.

1.2.2. Pharmacokinetics of NC318: Clinical Data

Patient PK data was recently analyzed and profiled from the ongoing Phase 1 (n=49) and Phase 2 (n=38) studies. NC318 was measured in serum as a concentration in µg per mL. Generable (www.generable.com, Long Island City, NY 11101) applied Bayesian hierarchical modeling and calculated a T1/2 alpha of NC318 of 5.08 hours and a T1/2 beta of 52.46 hours, suggesting more frequent dosing to increase exposure.

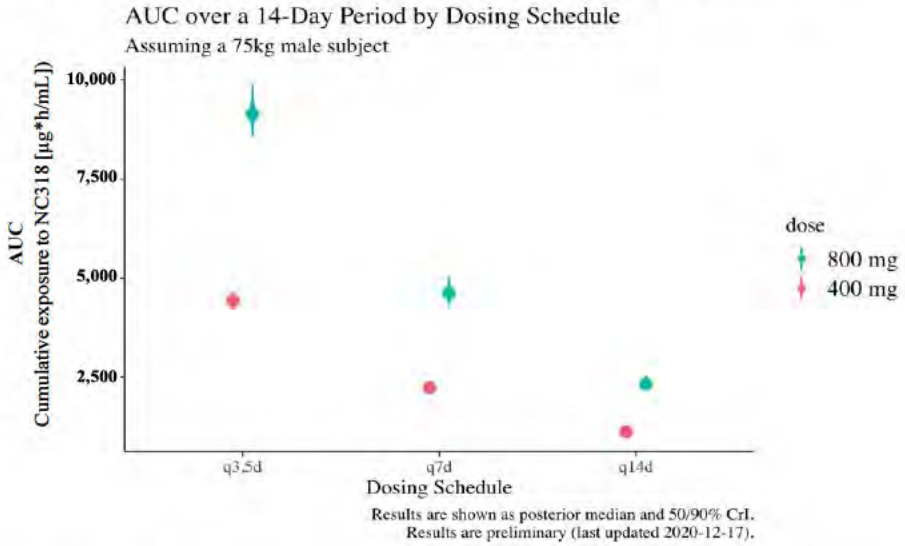
We also observed an increase in a soluble form of Siglec-15 (S15) in all patients receiving NC318 treatment that was dose dependent.. S15 levels peaked at 24 hours post NC318 treatment and reduced to close to baseline levels by Day 8 (Figure 1). Furthermore, soluble S15 level appears to settle after week 8.

Figure 1: Soluble Siglec-15 Increases at 24 hours and Reduces to Lower Levels onDay 8



Further PK/PD modeling by Generable using the current 400 mg Q2W shows that the drug exposure (AUC, cumulative exposure to NC318) of 800 mg weekly (Q1W) appears to be nearly ten-fold higher in exposure and is equivalent to 400 mg twice a week (Figure 2).

Figure 2: NC318 Area Under the Curve (AUC) by Dosing Schedule



Abbreviation: AUC = area under the curve.

Logistically, 800 mg Q1W is more feasible than 400 mg twice a week (2x/week) to achieve a higher overall exposure. In addition, in the Phase 1 portion of the NC318 study, 4 patients received 800 mg every 2 weeks (Q2W). Their serum NC318 levels on Day 8 were at 1.8 ± 1.4 µg/mL, below the estimated EC_{30} of 7 µg/mL, suggesting it is safe to give another dose of NC318 on Day 8.

1.2.3. Pharmacology of NC318

The pharmacology of NC318 has been studied in a variety of *in vitro* and *in vivo* systems to support its use as an investigational drug in oncology.

NC318 binds to human S15 with an affinity of 0.36 nM. Flow cytometer analysis of NC318 binding to various cells stably transduced or transiently transfected with human, cynomolgus monkey, rat and mouse S15 showed an effective concentration for 50% of maximum effect (EC_{50}) that ranged from 0.4 to 2.4 nM. U87 human glioma cells that naturally express low levels of S15 revealed an EC_{50} for NC318 with a binding affinity of approximately 53 nM.

Modest antibody dependent cellular cytotoxicity (ADCC) activity was observed against Raji.hS15 cells, which express significantly higher levels of S15 than those expected on most tumors. No complement dependent cytotoxicity (CDC) activity was observed. *In vitro* assays were also conducted to evaluate risk of cytokine release syndrome (CRS). Using conditions that would have predicted the high risk of CRS for the anti-CD28 mAb TGN1412 (Stebbings 2007; Findlay 2010), NC318 did not promote cytokine release relative to a negative control (anti-RSV) (Synagis®). Mouse NC318 also demonstrated modest effects on blocking mouse osteoclast formation of RAW264.7 cells *in vitro*, suggesting that the drug may reduce bone loss *in vivo*.

NC318 has been tested in cell-based assays using human peripheral blood mononuclear cells (PBMC). Expression of S15 in healthy human PBMC is limited. Assays using exogenous S15 fusion protein have been established to assess the activity of NC318. NC318 blocks S15 fusion protein mediated effects on primary human myeloid cells and T cells. THP-1, a human myeloid cell line, was stably transduced with S15 to further test NC318. The THP-1.hS15 cells highly express S15. NC318 demonstrated blocking of the immunosuppressive effect mediated by S15 expressed in these myeloid cells. NC318 was tested in a co-stimulation assay with healthy human PBMC. NC318 dose dependently increased IL-2 secretion in the presence of anti-CD3/SEB co-stimulation of PBMC from approximately 50% of the tested donors screened.

In vivo, mouse NC318 was effective as a monotherapy in three subcutaneous (SC) tumor models and one lung metastasis model. These models may be relevant to the subset of human cancers with $S15^+$ tumor cells or $S15^+$ M2 macrophages. Mouse NC318 treatment increased CD8 T cell and tumor specific CD8 T cell infiltration into the tumors and reduced the number of macrophages in the TME. Mouse NC318 combined with PD-1 blockade showed enhanced anti-tumor activity in a SC tumor model. Mouse NC318 can enhance an LPS-mediated immune response in wild type mice that is line with the outcome from LPS challenged S15 KO mice.

1.2.4. Non-Clinical Safety and Potential Risks of NC318

The toxicology of NC318 has been studied in a variety of *in vitro* and *in vivo* systems to support its use as an investigational drug in oncology.

In a GLP-compliant repeat-dose toxicology study of NC318 in Sprague Dawley rats, 178 animals (87/sex) were randomly assigned to four groups. Each main study group that received 0, 3, 30 and 100 mg/kg of NC318 included 15/sex/group; 10/sex/group were sacrificed 4 days (SD 33) after the last dose and 5/sex/group were sacrificed following a 6-week recovery period (SD 75). Animals were subjected to a full gross necropsy on SD 33 (terminal) or SD 75 (recovery). The toxicokinetic (TK) satellite group included 9/sex/group with 3, 30 or 100 mg/kg of NC318 treatment; 3/sex/group were evaluated at each timepoint. Eight animals developed ADA against NC318 in the main & recovery animals that received NC318. Treatment with NC318 at doses up to 100 mg/kg had no effect on mortality, physical examinations, cage-side and post dose observations, body weights, body weight changes, food consumption, clinical pathology (clinical chemistry, hematology, coagulation, and urinalysis), gross pathology, absolute and relative organ weights, or histopathology. The NOAEL for NC318 following 5 weekly IV doses was 100 mg/kg in Sprague Dawley rats.

In a GLP-compliant repeat-dose toxicology study in cynomolgus monkeys, animals were administered with 0, 3, or 30 mg/kg NC318 (N=5/sex/group; 3/sex Main Group and 2/sex Recovery Group) on SD 1, 8, 15, 22, and 29 by 30-min IV infusion. Animals at the highest dose level received 100 mg/kg NC318 (N=5/sex) on SD 1 and 60 mg/kg/dose on SD 8, 15, 22, and 29. The dose level for the highest dose was lowered beginning with the second dose due to periorbital swelling observation in male animals and 2 female monkeys with severe toxicity following the first dose on SD 1. Animals were subjected to a full gross necropsy and histopathology analysis on SD 31 (terminal) or 71 (recovery). Three NC318 treated recovery monkeys developed neutralized antibody (nAb), which was associated with accelerated CL, and they were removed from the mean TK profiling. No adverse findings were associated with ADA positivity. At terminal necropsy, 1 out of 6 animals at 30 mg/kg dose and 2 out of 5 animals at 100/60 mg/kg/dose showed

microscopic changes in the kidneys that consisted of minimal degeneration/necrosis of renal tubular cells with intratubular neutrophilic inflammation and intratubular and/or tubular hemorrhage. Evidence of kidney injury was present at the end of the recovery period (6 weeks after the fifth dose) in 1 out of 3 animals at 100/60 mg/kg/dose; no animals (0 out of 4) at 30 mg/kg/dose displayed renal injury suggesting that glomerular findings at 30 mg/kg/dose was reversible. The cause of severe adverse effects of the 2 female animals on SD 1 after 100 mg/kg dose was undetermined but the same microscopic changes in the kidneys were observed. There was no evidence of any microscopic changes at the lowest dose (3 mg/kg). The microscopic changes in the kidney were in a dose dependent manner; however, they were not accompanied by any evident clinical findings. The NOAEL of NC318 was 3 mg/kg when administered five times over a four-week interval to male and female cynomolgus monkeys by 30-minute IV infusion. The MTD was 30 mg/kg.

1.3. Study Rationale

Immunotherapies have demonstrated an effective anti-tumor immune response and induce significant clinical benefit for a subset of patients with cancer. This study will evaluate a novel target, S15, which has been shown to mitigate immunosuppressive macrophages in the TME. Immunotherapies for cancer have shown great promise, however, there is still an unmet medical need for additional therapies. NC318 may be an effective immunotherapy for some cancer patients.

1.3.1. Rationale for the Safe Starting Dose

The safe starting dose (SSD) for NC318 is 8 mg (correlates to 0.1 mg/kg), and this was determined based on all relevant preclinical pharmacology and toxicology data, including dose-response in human cell-based assays, and anti-tumor effects in mouse tumor models.

NC318 has been well-tolerated and repeated administration of dosages 100-fold higher (on a mg/kg basis) than the planned clinical starting dose were not associated with any adverse toxicities.

A risk assessment for NC318 was performed to support dose selection. Relevant factors for NC318 are discussed below:

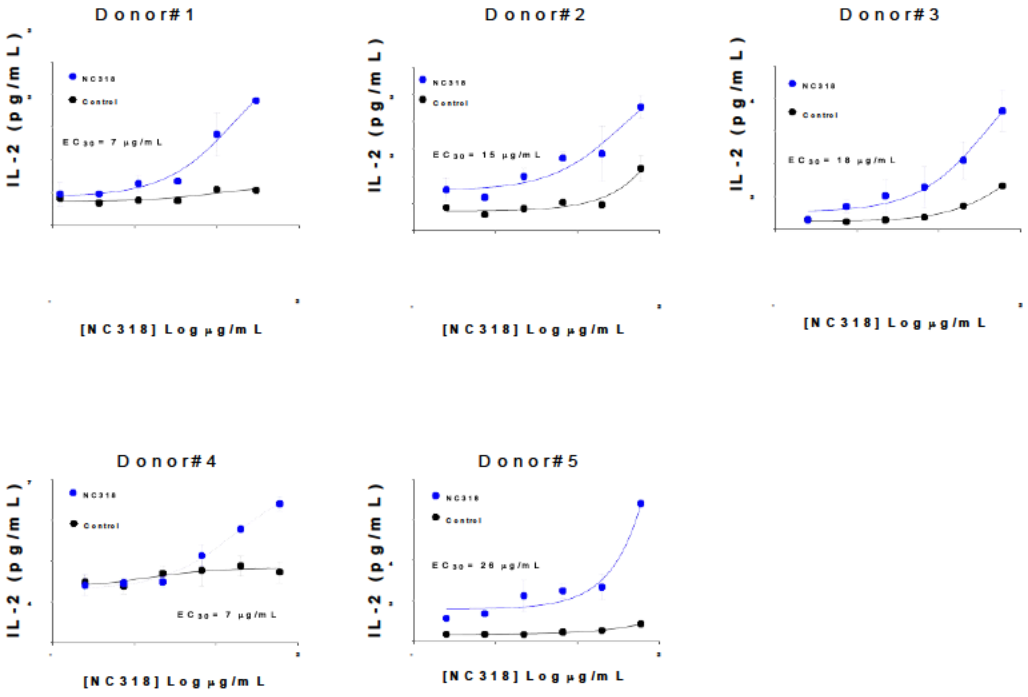
- *Mechanism of action:* NC318 does not directly activate T cells. NC318 did not promote cytokine release from PBMC, using assays that would have predicted the TGN1412 cytokine storms. NC318 may help to promote an effective anti-tumor immune response by two mechanisms. NC318 blocks the interactions between S15 and its binding partner on T cells or myeloid cells and releases S15-mediated suppression. NC318 may also bind to cells expressing high levels of S15 and mediate direct tumor killing.
- *Nature of the target:* S15 is not a master regulator of the immune system such as CD28 or CTLA-4. While CTLA-4 KO mice die 3-4 weeks after birth from lymphoproliferative disease affecting multiple organs, S15 KO mice developed without physical abnormalities other than slightly increased trabecular bone mass and mild osteopetrosis (Hiruma 2013).
- *Relevance of animal models*: S15 is conserved across mammals and NC318's parent, 5G12, is active in multiple species including rodents.

The rationale for the proposed Phase 1 SSD of 8 mg (0.1 mg/kg) is based on the collection of preclinical pharmacology and toxicology data, including calculated and experimentally assessed

dose-responses in human cell-based assays, and anti-tumor responses in syngeneic tumor models. The US FDA Guidance for Industry Estimating the Maximum Safe Starting Dose (SSD) in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers was used. The NOAEL determined in the GLP-compliant repeat-dose toxicity study in cynomolgus monkeys was 3 mg/kg (potentially greater than 3 mg/kg and less than 30 mg/kg). NC318 is pharmacologically active in rodent species and the NOAEL was 100 mg/kg in Sprague Dawley rats. Since NC318 is a protein administered via IV with a molecular weight > 100 kDa, mg/kg scaling was used, and the human-equivalent dose (HED) was determined to be 3 mg/kg. A default safety factor of 10 is appropriate, as there are no considerations indicating a safety concern that warrants increasing the safety factor. Based on this and additional analyses, an appropriate SSD would be 0.1 mg/kg, and this is consistent with additional considerations discussed below.

Animal toxicology data supported the calculation of an effective concentration for 30% of maximum effect (EC_{30}) as a target SSD. Adverse microscopic changes in the kidney were observed in cynomolgus monkeys in a dose dependent manner. There was no evidence of any microscopic changes at the lowest dose (3 mg/kg), and only 1 out of 10 animals had changes at 30 mg/kg (serum level at 800 µg/ml). Among the 10 animals exposed to the highest doses (100/60 mg/kg, serum levels at 2450 to 1550 µg/ml) 4 exhibited cases of microscopic changes during the acute phase and 1 case of microscopic changes after recovery. These microscopic changes were not accompanied by any evident clinical findings and occurred at dose levels above any dose expected to be tested in patients. Given the observed microscopic adverse renal changes the EC_{30} was selected to calculate the SSD. Relevant pharmacology data from an *ex vivo* co-stimulation assay, shown in Figure 3, were used to select the SSD. Primary cells from 10 healthy donors were tested, among which 5 donors responded to NC318 treatment in culture. Table 1 summarizes the HED from these 5 donors, which demonstrated an average EC_{30} of 15 µg/ml (a HED of 0.6 mg/kg) with a range of 7 – 26 µg/ml (HED 0.3 – 1.0 mg/kg). To insure optimal safety for the subjects, an SSD below the lowest HED was selected.

Figure 3: Pharmacology Data Set



Abbreviations: EC_{30} = effective concentration for 30% of maximum effect; IL = interleukin.

Table 1: Human-Equivalent Dose of EC30 from a Co-Stimulation PBMC Assay of NC318

	EC_{30} (µg/ml)	HED level (mg/kg)
Donor#1	7	0.3
Donor#2	15	0.6
Donor#3	18	0.7
Donor#4	7	0.3
Donor#5	26	1.0

Abbreviation: EC_{30} = effective concentration for 30% of maximum effect; HED = human-equivalent dose.

The NOAEL of NC318 in cynomolgus monkeys is 3 mg/kg, which is more than 10-fold higher than the planned clinical SSD of 0.1 mg/kg. The murine CT26 plus $S15^+$ M2 macrophage colon cancer model is the most sensitive model for assessing pharmacologic effect. 10 mg/kg administered via intraperitoneal (IP) injection was determined to be the lowest dose level that conferred a statistically significant improvement in tumor growth reduction or survival. For SSD estimation, the effective dose for 30% of maximum effect (ED_{30}) is estimated at 4.3 mg/kg; at this dose level, estimated circulating drug level is approximately 30 µg/ml; a human dose of 1.2 mg/kg is expected to provide similar drug exposure. Maximal efficacy was observed at a dose level of 30 mg/kg, corresponding to approximately 125 µg/ml murine NC318 (5G12) in serum, and an HED of 5 mg/kg.

Given the known biology of the S15 pathway and its relevance in immune function, immune related adverse events (irAE) are the most likely class of toxicity. This clinical protocol has been written to minimize and manage any potential irAEs and any infusion reactions. Eligibility criteria have been established to exclude subjects in the Phase 1 clinical study with a history of autoimmune disease or who may have experienced an unresolved irAE to another immunotherapy.

Based on analysis of all relevant pre-clinical pharmacology and toxicology parameters, 8 mg or 0.1 mg/kg has been selected as a starting dose, which is lower than the lowest observed EC_{30} and is less than 1/10 of the NOAEL. The proposed dosing scheme provides a starting dose that is expected to minimize safety risks. In the absence of dose-limiting toxicity, the maximum dose that may be explored in this trial will be 800 mg (10 mg/kg) - this is below the MTD in the GLP repeat dose tox (30 mg/kg) study in cynomolgus monkeys.

1.3.2. Rationale for Fixed Dosing

Fixed dosing has several advantages over weight-based dosing, including convenience of preparation and administration, reducing errors in preparation calculation, and minimization of drug waste. Body size-based dosing and fixed dosing of mAbs have been evaluated with the 2 dosing approaches performing similarly, with body size–based dosing not always offering an advantage in reducing variability of exposure. The authors of these studies concluded that either approach may be used in first-in-human (FIH) studies and that fixed dosing is recommended as the preferred approach because of the advantages mentioned above (Wang 2009; Bai 2012).

1.3.3. Rationale for Phase 2 Dose Selection and Administration Schedule

During Phase 1 Dose Escalation, various dose levels were evaluated ranging from 8mg to 1600mg. During the initial Phase 2 design, a RP2D of 400mg Q2W was tested. As stated in Section 1.2.2, PK/PD modeling shows initial weekly dosing of 800 mg will achieve adequate exposure of NC318. Upon further review of ongoing Phase 1/2 study, soluble S15 levels that increased within24 hours of NC318 infusion during Cycle 1 seemed to normalize after cycle 4 (~8 weeks). Furthermore, T cell activation appears critical only for the first 8 weeks of immune checkpoint therapies. Rizvi has shown that a patient responded for months after tumor-specific T cells decreased after 9 weeks (Rizvi 2015). Taken together, a new RP2D and administration schedule have been chosen for NC318. The new RP2D and administration schedule will be as follows:

- **Initial Dosing**: 800 mg weekly for 8 Cycles
- **Subsequent Dosing (Cycle 9 onwards)**: 800 mg every 2 weeks

Subjects will remain on the Subsequent Dosing Schedule of 800 mg every two weeks until disease progression, withdraw of consent, or intolerable toxicity (whichever comes first).

1.3.3.1. Phase 2 Safety Run-In Rationale

Based on PK/PD modeling and normalization of soluble S15, NC318 will be given at 800 mg Q1W for 8 cycles and subsequent cycles will be given at 800mg Q2W. During Phase 1 Dose Escalation (n=49), various dose levels were evaluated ranging from 8 mg to 1600 mg. There wereno DLTs at 800 mg Q2W. However, there was one DLT at 1600 mg Q2W as previously reportedduring annual reporting amongst the 4 patients treated in that dose escalation cohort. At 800 mg Q2W (n=4), only non-serious adverse events ≤ Grade 2 were reported.

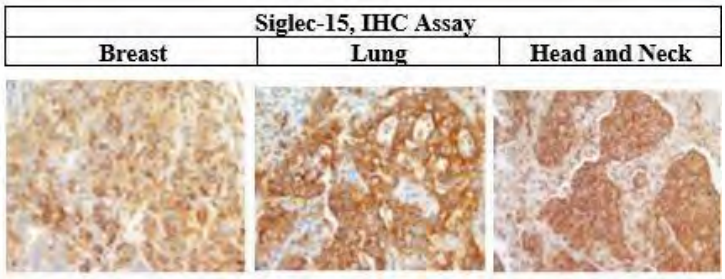
In accordance with the PK/PD data from the ongoing Phase 1/2, the RP2D has been adjusted to 800 mg with an alternative administration schedule. To evaluate the new RP2D and administration schedule, a Phase 2 Safety Run-In will be conducted utilizing a 3+3 design.

1.3.4. Rationale for Subject Population for Phase 2

To further elucidate the effects of NC318, 6 different tumor types were initially selected for investigation in Phase 2. HNSCC and NSCLC will be evaluated, because they are both immunogenic histologies that have been proven to respond to checkpoint inhibitors (CTLA-4 and PD-1). Included in the Investigational New Drug (IND) enabling studies, experiments looked at the expression of S15 on tumor samples from patients with a variety of tumor histologies including, but not limited to, NSCLC, TNBC, and ovarian cancer.

Previously the study was designed to enroll patients with low PD-L1 (<50% TPS) based on the notion of mutually exclusive expression of PD-L1 and siglec-15 as stated above due to the lack of standardized siglec-15 staining. However, the most recent CLIA validation study has validated adenocarcinoma of the lung, squamous cell carcinoma of head and neck, and breast cancers for siglec-15 staining in tumors and immune cell infiltrates in the stroma (Figure 4). Therefore, to elucidate the effects of NC318, 3 tumor types with CLIA validated siglec-15 positive staining, namely adenocarcinoma of the lung, squamous carcinoma of head and neck, and breast cancer, were selected.

Figure 4: S15 Expression in Breast, Lung, and Head and Neck Tumors



In addition, the following observations have been made based on the data obtained from the ongoing Phase 1/2 trial thus far:

1. Of the 4 responders (1 CR and 1 PR in NSCLC, 1 PR in HNSCC, and 1 PR in TNBC) of 96 evaluable subjects enrolled in Phase 1/2, 2 had TMB-H, and 1 had MSI/dMMR. Therefore, it is likely that either TMB-H and/or MSI/dMMR status is driving the T cell infiltration in the TME of those subjects to respond to NC318. Since all of those subjects were refractory to ICI therapies, we hypothesize that NC318 might have alleviated the immunosuppressive environment of the TME in those patients for the reactivation of T cells. Therefore, in cohort 1, we will include TMB-H and/or MSI/dMMR positive NSCLC, HNSCC, or Breast cancer, followed by selection of S15 expression in the TME.
    a. The basis for evaluating subjects based on S15 expression came from the data obtained in the ongoing Phase 1/2 study (Shum 2021). S15 expression confirmed by CLIA validated IHC staining was associated with 40% disease control (stable disease) compared to only 12% disease control in the S15- group.
2. Since TMB-H and MSI-H have been widely accepted predictors for immunotherapies, a separate cohort of TMB-H and/or MSI-H/dMMR tumors that do not fall under the criteria to be included in Cohort 1 has been added to the study. Upon enrollment, subjects will be stratified into two subgroups based on S15 expression (S15+ Cohort 2a and S15- Cohort 2b) to explore how vital S15 expression is in the TME.

1.3.5. Rationale for Efficacy Endpoints

Efficacy endpoints of this study are secondary and include objective response rate (ORR), duration of response (DoR), duration of disease control (DC), and progression-free survival (PFS) by investigator assessment based on Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 and modified RECIST (mRECIST).

RECIST v1.1 will be adapted to account for the unique tumor response characteristics seen with immunotherapy (Chiou 2015). Immunotherapeutic agents may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents and can manifest a clinical response after an initial increase in tumor burden or even the appearance of new lesions. Standard RECIST may not provide an accurate response assessment of immunotherapeutic agents. Therefore, RECIST v1.1 will be used with the following adaptations:

If radiologic imaging shows initial progressive disease, then tumor assessment should be repeated at least 4 weeks but no later than 6 weeks later to confirm disease progression with the option of continuing treatment while awaiting radiologic confirmation of progression.

In subjects who have initial evidence of radiological progression but are clinically stable as defined below, it is at the discretion of the treating physician whether to continue a subject on study drug until repeat imaging is obtained. This clinical judgment decision should be based on the subject's overall clinical condition, including performance status, clinical symptoms, and laboratory data. Subjects may receive treatment while waiting for confirmation of disease progression if they are clinically stable as defined by the following criteria:

- Absence of clinically significant signs and symptoms indicating disease progression
- No decline in Eastern Cooperative Oncology Group (ECOG) performance status (Oken 1982)
- Absence of rapid progression of disease
- Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention

If repeat imaging shows < 20% tumor burden compared with nadir, stable or improved previous new lesion (if identified as cause for initial disease progression), and stable/improved nontarget disease (if identified as cause for initial disease progression), then treatment may be continued or resumed. If repeat imaging confirms disease progression due to any of the scenarios listed below, then subjects will be discontinued from study therapy. However, if a subject has confirmed radiographic progression (i.e., 2 scans at least 4 weeks but no later than 6 weeks apart demonstrating progression) per mRECIST but the subject is achieving a clinically meaningful benefit, then an exception to continue treatment may be considered after consultation with the medical monitor. Clinically stable subjects at the confirmatory scan should also have no further increase in the target lesions, no unequivocal increase in nontarget lesions, and no additional new lesions develop (non-worsening disease progression) to continue study treatment.

In determining whether the tumor burden has increased or decreased, site study teams should consider all target lesions as well as nontarget lesions (refer to RECIST v1.1 guidelines).

Scenarios where disease progression is confirmed at repeat imaging include the following:

- Tumor burden remains ≥ 20% and at least 5 mm absolute increase compared with nadir
- Nontarget disease resulting in initial disease progression is worse (qualitative)
- New lesion resulting in initial disease progression is worse (qualitative)
- Additional new lesion(s) are found since the last evaluation

When feasible, subjects should not be discontinued until progression is confirmed. This allowance to continue treatment despite initial radiologic progression considers the observation that some subjects can have a transient tumor flare in the first few months after the start of immunotherapy, but with subsequent disease response. Subjects who are deemed clinically unstable are not required to have repeat imaging for confirmation of disease progression.

1.4. Summary of Clinical Experience

The Phase 1 component demonstrated that NC318 is safe and tolerable when administered as a monotherapy at doses ranging from 8 mg to 800 mg. In order to seek optimal benefit for potential patients, a revised dosage and administration schedule will be explored using an updated Phase 2 design. Based on the results of the amended design, the optimal dosing strategy will be used for subsequent clinical trials. During the ongoing Phase 2 component of the study, analysis of PK and PD has revealed that doses ranging from 240 mg to 1600 mg every 2 weeks are pharmacologically active.

1.4.1. Ongoing Clinical Study NC318-01

The FIH clinical trial, Study NC318-01 completed Phase 1 enrollment with 49 subjects dosed in 15 tumor types. Subjects received NC318 every two weeks at doses of 8, 24, 80, 240, 400, 800, and 1600 mg. NC318 was well tolerated at doses up to 800 mg every two weeks and expanded cohorts of subjects were further evaluated at the 80, 240, and 400 mg dose levels. One Phase 1a subject enrolled in Cohort 6 (1600 mg) with Grade 3 pneumonitis died of respiratory failure due to healthcare acquired pneumonia on 28-SEP-2019 and no further subjects were enrolled in the 1600mg cohort (n=4). As such, 400 mg every 2 weeks was determined as the original recommended Phase 2 dose.

As of the data cutoff date of 29-SEP-2021, the treatment-related adverse events (TRAEs) of any grades experienced by more than 5% of patients in Phase 1 (n=49) were pruritis (16.3%), diarrhea (16.3%), were infusion related reactions (10.2%), fatigue (8.2%), elevated amylase (8.2%), elevated lipase (8.2%), muscular weakness (8.2%), and headaches (6.1%). In Phase 1, there was 1 case of Grade 3 increased amylase, 3 cases of increased lipase {Grade 3 (n=2), Grade 4 (n=1)}, and 1 case of Grade 3 Muscle Weakness. TRAEs of any grade experienced by more than 5% of patients in Phase 2 (n=47) were infusion reaction (19.1%), pruritis (8,5%), chills (8.5%), influenza-like illness (6.4%), and nausea (6.4%). In Phase 2, there was 1 Grade 3 Infusion reaction.

From clinical benefit aspect from Phase 1 and 2 studies of NC318, there were 1 CR in NSCLC, 3 PR (1 each in NSCLC, HNSCC and TNBC) and 28 SD (stable disease), with total disease control rate of 37% and median progression free survival of 5.0 months (Shum, 2021). One subject with NSCLC in CR remains on study more than 3 years. One other subject with NSCLC in PR elected to discontinue study treatment after nearly 3 years on study. Furthermore, of the 4 responders, 1 had MSI, and 2 had TMB-H, but no tumor abnormality data is available from the CR subject.

Additional details regarding clinical experience with NC318 are available in the Investigator's Brochure.

1.5. Research Hypotheses

NC318 will be adequately tolerated following administration in multiple ascending doses to subjects with refractory solid tumors and that such administration may result in clinical benefit.

2. OBJECTIVES AND ENDPOINTS

2.1. Objectives

2.1.1. Primary Objectives

1) To evaluate the safety, tolerability, and dose-limiting toxicities (DLTs) of NC318
2) Define an MTD or PAD of NC318 in subjects with advanced or metastatic solid tumors

2.1.2. Secondary Objectives

1) To evaluate the PK profile of NC318
2) To assess preliminary efficacy of NC318 by assessing ORR, DoR, and disease control rate (DCR) per RECIST v1.1
3) To evaluate expression of Siglec-15 in tumor tissue and immune cell infiltrates at baseline and following NC318 treatment, and correlate expression level with efficacy and changes in tumor infiltrating lymphocytes

2.1.3. Exploratory Objectives

1) To assess the immunogenicity of NC318
2) To explore biomarkers that may predict the pharmacologic activity of NC318
3) To characterize the effect of NC318 on immune markers such as cytokine and immune cell phenotypes
4) Soluble S15 level at baseline and changes after NC318 treatment, and correlation with efficacy.

2.2. Study Endpoints

2.2.1. Primary Endpoints

1) Safety and tolerability will be assessed by monitoring frequency, duration, and severity of adverse events (AEs). Note: Toxicity grading per NCI CTCAE v5.0
2) Define an MTD or PAD of NC318 in subjects with advanced or metastatic solid tumors

2.2.2. Secondary Endpoints

1) The endpoints for assessment of PK of NC318 include individual NC318 concentrations in serum and PK parameters.
2) The endpoints for assessment of antitumor activity/efficacy include objective response (OR) and DC based on RECIST v1.1, DoR, PFS, and overall survival (OS) as per RECIST v1.1
3) To evaluate expression of Siglec-15 in tumor tissue and immune cell infiltrates at base line and following NC318 treatment, and correlate expression level with efficacy and changes in tumor infiltrating lymphocytes

2.2.3. Exploratory Endpoints

1) Immunogenicity, defined as the occurrence of ADA to NC318 will be determined.
2) Biomarker effects of NC318 in peripheral blood and tumor tissue will be assessed, including but not limited to the following:
    - Whole blood immune cell population profiling/immune-phenotyping.
    - Plasma markers of inflammation or immune modulation (cytokine levels).
    - Soluble S15 level at baseline and changes after NC318 treatment, and correlation with efficacy.
    - The expression of additional biomarkers may also be assessed.

3. STUDY DESIGN

3.1. Description of the Study

This is an open-label, nonrandomized, Phase 1/2 study to determine the safety and tolerability, define the MTD or PAD, and to assess the preliminary efficacy of NC318 in subjects with advanced or metastatic solid tumors. Subjects will receive NC318 on Day 1 of each cycle. Phase 1 of the study will begin with 14-day cycles; however, alternate dose administration schedules may also be explored depending on PK, PD, biomarker, safety results, and feedback from investigators.

The study will be conducted in 2 parts:

- **Phase 1 – Dose Escalation and Safety Expansion** will determine the PAD, defined as a dose that provides a maximal biologic effect, such as an increase in biomarkers of immune activation or reduction of markers associated with immunosuppression, and/or the MTD of NC318, including defining the optimal dose administration schedule and the maximum number of tolerated doses (MNTD). **Phase 1 enrollment of the study is complete.**
- **Phase 2 – Safety Run-In and Dose Expansion** will further evaluate the safety, tolerability, preliminary efficacy, and PK/PD activity of NC318. **The study is currently enrolling Phase 2.**

3.1.1. Phase 1

3.1.1.1. Phase 1a - Dose Escalation

In Phase 1a, subjects with advanced or metastatic solid tumors who progressed after treatment with available therapies that are known to confer clinical benefit, who are intolerant to treatment, or refuse standard treatment were enrolled. A 3 + 3 design with escalating dose levels were explored to determine the MTD and PAD of NC318. The cohorts and dose levels evaluated during Phase 1 are shown in Table 2.

Table 2: NC318 Dose Levels and Cohorts

Cohort	Dose of NC318
-1 (Starting dose)	8 mg
1	24 mg
2	80 mg
3	240 mg
4	400 mg
5	800 mg
6	1600 mg

The Phase 1a design is summarized below:

A minimum of 3 evaluable subjects will be enrolled in each cohort beginning with Cohort -1 (8 mg; starting dose). The first 3 evaluable subjects within a cohort will be observed for a DLTobservation period of 28 days, before the next cohort begins enrollment. Only one subject can be dosed on the first day of dosing for each cohort (additional subjects can begin dosing in ≥ 48 hours). The dose will be escalated if 0 of the first 3 evaluable subjects enrolled has a DLT. If 1 of the first 3 evaluable subjects enrolled has a DLT, then the cohort will be expanded to include 3 additional evaluable subjects, and if no DLT occurs in the additional 3 subjects, then the dose will be escalated. If a DLT occurs in one-third or more of the expanded cohort, then the MTD will be deemed to be exceeded and the previous dose level will be considered the MTD. Intermediate dose levels below the MTD may be explored pending safety review. If only 3 subjects were treated at the MTD or PAD, then a minimum of 3 additional evaluable subjects will be enrolled at this dose before it is administered in Phase 2 of the study.

If Cohort -1 (8 mg; starting dose) exceeds the MTD, the sponsor and investigators will consider dosing NC318 at a lower dose, and/or investigate 8 mg at alternate dose schedules, based on available safety, PK, PD, and biomarker data. If an alternate schedule is determined to be safe, re-escalation of NC318 will proceed according to Table 2.

Throughout the treatment period, if > 33% of subjects (a minimum of 6 subjects) experience a ≥ Grade 3 toxicity related to study drug after completing ≥ 4 cycles, then dose administration will be stopped, and the MNTD will be determined in conjunction with the investigators and sponsor based on all available safety data.

Additional subjects will be enrolled in a cohort to achieve the minimum of 3 evaluable subjects. Subjects who drop out for reasons other than a DLT (e.g., events clearly associated with the underlying disease, disease progression, concomitant medication, or comorbidity), during the 28-day DLT observation period will be considered nonevaluable and will be replaced. Dose modifications should not be made during the DLT observation period without discussion with the medical monitor. Intrasubject dose escalation is not permitted; however, once the RP2D and schedule has been determined, ongoing subjects in Phase 1 may be permitted to escalate to the RP2D with approval of the medical monitor.

3.1.1.2. Phase 1b – Safety Expansion

The purpose of Phase 1b was to evaluate additional PD activity of NC318 and confirm the preliminary safety of the dose escalation cohorts from Phase 1a. Several cohorts from Phase 1a were expanded at doses and schedules equivalent to or lower than the highest dose levels determined to be safe and/or doses determined to be pharmacologically active. All doses and schedules explored during the safety expansion will depend on PK, PD, biomarker, and safety results.

The following cohorts were expanded during Phase 1b: Cohort 2 (80 mg), Cohort 3 (240 mg), and Cohort 4 (400 mg). Phase 1 enrollment of the study is complete. A total of 49 subjects were enrolled across Phase 1a and Phase 1b.

The Phase 1a design is summarized below:

Approximately 36 evaluable subjects will be enrolled in the Phase 1b safety expansion, with each cohort enrolling approximately 9 evaluable subjects. If < 3 of 9 evaluable subjects experience a DLT, the cohort will be deemed safe. If > 1 safety expansion cohort is deemed safe, then a recommended dose and schedule will be determined in conjunction with the investigators and sponsor based on all available safety, PK, PD, and biomarker results. The safety expansion cohorts may be conducted in parallel to Phase 2 and may be limited by the sponsor to subjects with specific tumor types to achieve a balance across cohorts.

3.1.2. Phase 2

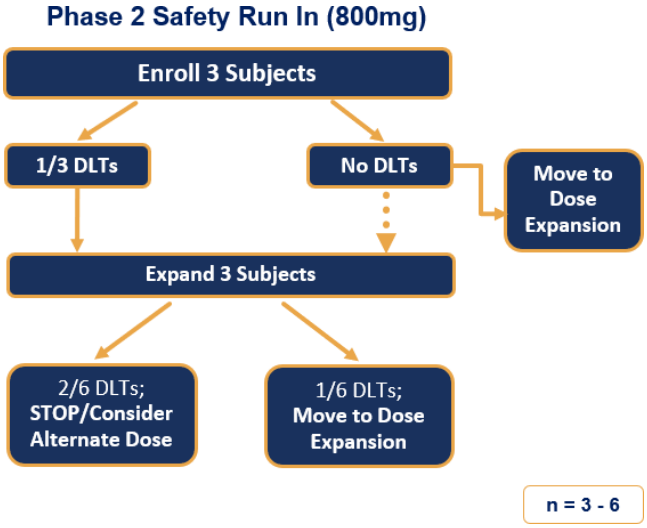
The trial is currently enrolling Phase 2. Phase 2 will evaluate subjects with advanced or metastatic solid tumors characterized as Tumor Mutational Burden High (TMB-H) and/or MicroSatellite Instability High (MSI-H)/ deficient MisMatch Repair (dMMR). The following tumor types will be enrolled: Lung, HNSCC, Breast, Endometrial, Melanoma, CRC, Urothelial, Cholangiocarcinoma, and other tumors known to be TMB-H and/or MSI-H/dMMR.

Previously as part of the initial Phase 2 design, a RP2D of 400 mg Q2W was tested. 47 subjects were treated at the initial RP2D. Based on further evaluation of the PK/PD studies from the original Phase 1/2, the RP2D was adjusted to 800 mg with an alternative administration schedule. To further evaluate the new RP2D, a Phase 2 Safety Run-In will be conducted utilizing a 3+3 design (Figure 5).

3.1.2.1. Phase 2 Safety Run-In to Evaluate New RP2D

The first 3 evaluable subjects will be observed for a DLT observation period of 28 days. Only one subject will be dosed on the first day of dosing (additional subjects can begin in ≥ 48 hours). If there are no DLTs observed in the first 3 subjects, then Phase 2 Dose Expansion may begin at the new RP2D. If 1 of the first 3 evaluable subjects enrolled has a DLT, then the cohort will be expanded to include 3 additional evaluable subjects. If there are no DLTs in the additional 3 subjects, then Phase 2 Dose Expansion can begin at the new RP2D. If a DLT occurs in one-third or more of the expanded cohort, then enrollment will be paused and intermediate dose levels or alternative dose schedules may be considered.

Figure 5: Phase 2 Safety Run-In Design (3+3)



Abbreviation: DLT = dose-limiting toxicity

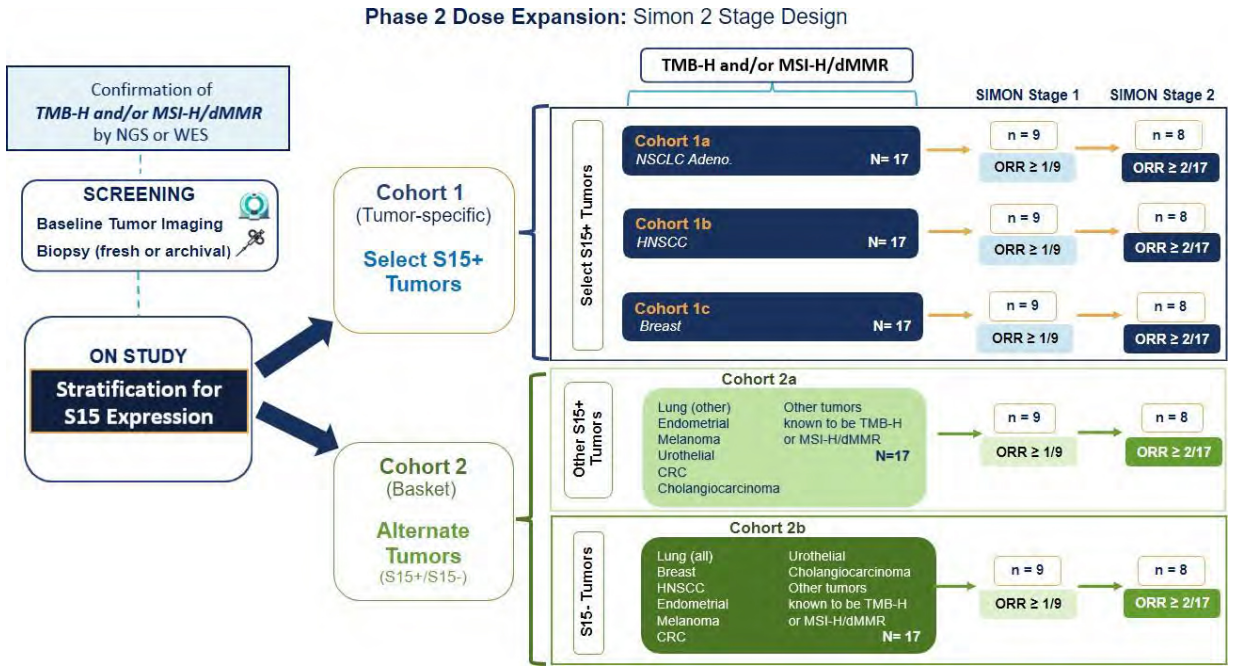
3.1.2.2. Phase 2 Dose Expansion at New RP2D

Phase 2 Dose Expansion will further evaluate the safety, tolerability, preliminary efficacy, and PK/PD activity of NC318 at the RP2D utilizing a Simon 2-stage design (Figure 6: Phase 2 Dose Expansion Design (Simon 2-Stage)

). Subjects will be stratified after enrollment into two main cohorts based on S15 expression and tumor type:

- **Cohort 1**: Select S15+ Tumors (Lung (adenocarcinoma), HNSCC, Breast)
- **Cohort 2**: Alternate Tumors (including Other S15+ Tumors and S15- Tumors)

Figure 6: Phase 2 Dose Expansion Design (Simon 2-Stage)



Abbreviations: CRC = colorectal cancer; dMMR = deficient DNA mismatch repair; MSI = microsatellite instability high; NGS = next generation sequencing; NSCLC = non-small cell lung cancer; ORR = objective response rate; TMB-H = Tumor mutational burden high; WES = whole exome sequencing.

Cohort 1: Select S15+ Tumors

This cohort will include subjects who are 1) confirmed as TMB-H and/or MSI-H/dMMR, 2) express S15, and 3) have one of the following tumor types, separated into tumor-specific subgroup:

- **Cohort 1a:** NSCLC (adenocarcinoma)
- **Cohort 1b:** HNSCC
- **Cohort 1c:** Breast cancer

Each subgroup will be independently assessed, guided by the Simon 2-stage design, and will enroll 9 evaluable subjects in Stage 1. If no responses are observed within a subgroup, then the sub-group will be discontinued. If 1 or more response are observed, Stage 2 will begin and enroll 8 additional evaluable subjects, for a total of 17 evaluable subjects within each subgroup (1a, 1b, and 1c).

Cohort 2: Alternate Tumors (including Other S15+ Tumors and S15- Tumors)

This basket cohort will include all other enrolled subjects who are confirmed as TMB-H and/or MSI-H/dMMR but do not fall under the criteria to be included in Cohort 1. Based on results of S15 expression, subjects will be further stratified into two subgroups:

- **Cohort 2a:** Other S15+ Tumors
- **Cohort 2b:** S15- Tumors

Each subgroup will be independently assessed, guided by the Simon 2-stage design, and will enroll 9 evaluable subjects in Stage 1. If no responses are observed within a subgroup, then the sub-group will be discontinued. If 1 or more response are observed, Stage 2 will begin and enroll 8 additional evaluable subjects, for a total of 17 evaluable subjects within each subgroup (2a and 2b).

3.2. Measures Taken to Avoid Bias

This is an open-label study; no formal comparisons will be made between subjects or against historical controls. Measurements of safety and efficacy are objective measurements, and only comparisons to pretreatment conditions will be made.

3.3. Number of Subjects

3.3.1. Planned Number of Subjects

Up to approximately 187 evaluable subjects may be enrolled in this study.

- Phase 1a Dose Escalation and Phase 1b Safety Expansion – 49 subjects enrolled
- Previous Phase 2 Design – 47 subjects enrolled
- New RP2D Phase 2 Safety Run-in – Up to 6 evaluable subjects
- New RP2D Phase 2 Dose Expansion – Up to 85 evaluable subjects

  Note: Number of planned subjects includes actual enrollment to date under previous protocol versions/amendments (during Phase 1 and previous Phase 2) and expected enrollment under revised Phase 2 design.

3.3.2. Replacement of Subjects

Subjects may be replaced for any of the following reasons:

- In Phase 1 and Phase 2, any subject who withdraws from treatment before the completion of the DLT period for any reason other than a DLT (is not evaluable for DLTs)
- A subject who is scheduled to have a biopsy for the purpose of this study and it is subsequently determined that tumor tissue cannot safely be obtained
- Subject does not meet the eligibility requirements of the study (accidental enrollment)

3.4. Duration of Treatment and Subject Participation

After signing the main informed consent form (ICF), screening assessments may be completed over a period of approximately 30 days.

Subjects will continue to receive NC318 until disease progression, withdrawal of consent, or intolerable toxicity (whichever comes first). After completing 12 months of treatment, if the subject is deriving benefit from NC318 and has not met any of the protocol defined conditions for withdrawal or discontinuation, the subject will be followed by the PI per standard of care. Subjects approved to continue treatment will remain on dose level assigned at the time of enrollment (unless otherwise approved for dose modification by the Sponsor). Safety reporting, documentation of disease assessments, and collection of survival status will continue per protocol.

3.5. Overall Study Duration

The study begins when the first subject signs the ICF. The end of the study will occur when all subjects have completed the last study follow-up visit or have discontinued study drug and have completed applicable follow-up assessments.

The end of the study may be declared when no more than 5 subjects remain on study drug for at least 6 months, at which point a database lock of the study may occur to allow for analysis of the study data. Any remaining subjects may continue to receive study drug and be seen by the investigator per standard of care. The investigator will be expected to monitor for and report any serious AEs (SAEs) and pregnancies, as detailed in Sections 8.3 and 8.5. The remaining subjects will be considered on study until a discontinuation criterion is met and written notification is provided to the sponsor.

3.6. Study Termination

The investigator retains the right to terminate study participation at any time, according to the terms specified in the study contract. The investigator is to notify the institutional review board (IRB)/independent ethics committee (IEC) in writing of the study's completion or early termination, send a copy of the notification to the sponsor or sponsor's designee, and retain 1 copy for the site study regulatory file.

The sponsor may terminate the study electively, if required by regulatory decision or upon review of emerging data. If the study is terminated prematurely, then the sponsor or designee will notify the investigators, the IRBs and IECs, and regulatory bodies of the decision and reason for termination of the study.

4. SUBJECT ELIGIBILITY

Deviations from eligibility criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability, and/or subject safety. Therefore, adherence to the criteria as specified in the Protocol is essential.

4.1. Subject Inclusion Criteria

A subject who meets all the following criteria may be included in the study:

1) Men and women aged 18 or older.
2) Willing and able to provide written informed consent for the study.
3) ECOG performance status 0 to 1.
4) Subjects with advanced unresectable and/or metastatic solid tumors confirmed to be TMB-H and/or MSI-H/dMMR including: Lung, Breast, HNSCC, Endometrial, Melanoma, CRC, Urothelial, Cholangiocarcinoma, and other tumors known to be TMB-H or MSI-H/dMMR.
5) Subjects who have disease progression after treatment with available therapies that are known to confer clinical benefit, or who are intolerant to treatment, or who refuse standard treatment.

   Note: There is no limit to the number of prior treatment regimens.

6) Presence of measurable disease based on RECIST v1.1. Tumor lesions situated in a previously irradiated area, or in an area subjected to other locoregional therapy, are not considered measurable unless there has been demonstrated progression in the lesion.

7) Able to provide pretreatment tumor tissue sample (archival ≤ 5 years old) or undergo tumor biopsy at Screening (must allow for adequate sample of tissue from appropriate site).

   Note: Formalin-fixed, paraffin embedded (FFPE) tissue blocks are preferred to slides. Newly obtained biopsies are preferred to archived tissue.

8) Female subjects of childbearing potential (defined as female subjects who have not undergone surgical sterilization with a hysterectomy and/or bilateral oophorectomy and are not postmenopausal, defined as ≥ 12 months of amenorrhea not caused by reversible conditions, diseases, or medications) and non-sterilized male subjects must agree to take appropriate precautions to avoid pregnancy or fathering children (with at least 99% certainty) from screening through 90 days after the last dose of study drug.

   Note: Females subjects of childbearing potential must have negative serum pregnancy test at screening.

4.2. Subject Exclusion Criteria

A subject who meets any of the following criteria will be excluded from the study:

1) Inability to comprehend or unwilling to provide informed consent.
2) Laboratory and medical history parameters not within the Protocol-defined range.
   a. Absolute neutrophil count < 1.5×10^9/L.
   b. Platelets < 100×10^9/L.
   c. Hemoglobin < 9 g/dL or < 5.6 mmol/L.
   d. Serum creatinine > 1.5 × institutional upper limit of normal (ULN) OR measured or calculated creatinine clearance < 30 ml/min for subjects with creatinine levels <1.5 x institutional ULN.
   e. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) > 2.5 × ULN with the following exceptions: Subjects with documented liver metastases: ALT, AST, and alkaline phosphatase ≤ 5 × ULN. Subjects with documented bone metastases: alkaline phosphatase ≤ 5 × ULN.
   f. Total bilirubin > 1.5 × ULN. **Note**: Subjects with documented Gilbert's syndrome with elevated baseline total bilirubin ≤ 3.0 mg/dL may be enrolled.
   g. International normalized ratio (INR)/prothrombin time (PT) > 1.5 × ULN or activated partial thromboplastin time (aPTT) > 1.5 × ULN; applies only to subjects who do not receive therapeutic anticoagulation; subjects receiving therapeutic anticoagulation should be on a stable dose.

3) Transfusion of blood products (including platelets or red blood cells) or administration of colony-stimulating factors (including granulocyte colony-stimulating factor, granulocyte macrophage colony–stimulating factor, or recombinant erythropoietin) within 7 days prior to first administration of study drug.

4) Receipt of anticancer medications or investigational drugs within the following intervals before the first administration of study drug:

   a. ≤ 14 days for chemotherapy, targeted small molecule therapy, or radiation therapy. Subjects must not have had radiation pneumonitis because of a treatment. A 1-week washout is permitted for palliative radiation to non–central nervous system (CNS) disease provided recovery is adequate.

      Note: Bisphosphonates and denosumab are permitted medications.

   b. ≤ 28 days for prior immunotherapy or persistence of active cellular therapy(e.g., chimeric antigen receptor T cell therapy; other cellular therapies must be discussed with the medical monitor to determine eligibility).

   c. ≤ 28 days for a prior mAb used for anticancer therapy except for denosumab.

   d. ≤ 7 days for immune-suppressive–based treatment for any reason.

      Note: Use of inhaled or topical steroids or corticosteroid use for radiographic procedures is permitted.

      Note: The use of corticosteroids equivalent to prednisone ≤ 10mg/day is allowed.

   e. ≤ 28 days or 5 half-lives (whichever is longer) before the first dose for all other investigational study drugs or devices.

   f. ≤ 14 days for a COVID-19 vaccine. **Note**: For 2-dose COVID-19 vaccines, subjects must wait at least 14-days after 2nd dose administration.

5) Has not recovered to ≤ Grade 1 from toxic effects of prior therapy (including prior immunotherapy) and/or complications from prior surgical intervention before starting therapy.

   Note: Subjects with stable chronic conditions (≤ Grade 2) not expected to resolve (such as neuropathy and alopecia) are exceptions and may enroll.

   Note: Subjects with a history of any grade immune-related ocular AE (e.g., episcleritis, scleritis, uveitis) will be excluded.

   Note: Subjects with a history of a Grade 3 or higher irAE from prior immunotherapies are excluded from the Phase 1a dose-escalation portion of the study.

6) Receipt of a live vaccine within 30 days of planned start of study therapy.

   Note: Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox/zoster, yellow fever, rabies, Bacillus Calmette–Guérin, and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines and are not allowed.

7) Active autoimmune disease that required systemic treatment in the past (i.e., with use of disease-modifying agents, corticosteroids, or immunosuppressive drugs).

   Note: Subjects with hyperthyroidism or hypothyroidism, who have not required any systemic treatment for an autoimmune disease for at least 2 years and have normal thyroid function or are stable on thyroid hormone replacement are allowed to participate in the study.

   Note: Replacement and symptomatic therapies (e.g., levothyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) are not considered a form of systemic immune suppressive therapy and are allowed.

8) Known active CNS metastases and/or carcinomatous meningitis.

   Note: Subjects with previously treated brain metastases may participate provided they are stable (without evidence of progression by imaging for at least 28 days before the first dose of study drug and any neurologic symptoms have returned to baseline), have no evidence of new or enlarging brain metastases or CNS edema, and have not required steroids for at least 7 days before the first dose of study drug.

9) Known additional malignancy that is progressing or requires active treatment, or history of other malignancy within 2 years of study entry after treatment with curative intent.

   Note: Cured basal cell or squamous cell carcinoma of the skin, superficial bladder cancer, prostate intraepithelial neoplasm, carcinoma in situ of the cervix, or other noninvasive or indolent malignancy, or cancers from which the subject has been disease free for > 1 year are not considered exclusionary.

10) Has a history of (non-infectious) pneumonitis / interstitial lung disease that required steroids or has current pneumonitis / interstitial lung disease.

11) Documented known activating or driver mutations (i.e. EGFR mutations/amplification, BRAF mutations, ALK alterations, etc.) which have not been previously treated with a standard of care targeted therapy.

12) Subjects with screening QTc interval > 470 milliseconds (corrected by Fridericia) are excluded.

13) Uncontrolled systemic fungal, bacterial, viral, or other infection despite appropriate anti-infection treatment.

14) Evidence of hepatitis B virus (HBV) or hepatitis C virus (HCV), unless the hepatitis is considered to be cured.

    Note: Subjects with no prior history of hepatitis B infection who have been vaccinated against hepatitis B and who have a positive antibody against hepatitis B surface antigen test as the only evidence of prior exposure may participate in the study.

    Note: If HCV/HBV antibody or antigen tests are positive, reflex testing (HCV RNA and/or HBV DNA tests) should be performed prior to assessing subject eligibility.

    Note: Subjects with prior hepatitis B virus [HBV] infection must have HBV viral load [VL] < 100 IU/mL before study enrollment and must be treated according to local standards;

hepatitis C virus [HCV] infection must have, before study enrollment, no detectable VL and must be treated according to local standards.

15) Known history of HIV (HIV 1 or HIV 2 antibodies).

16) Known allergy or reaction to any component of study drug or formulation components.

17) Is pregnant or breastfeeding or expecting to conceive or father children within the projected duration of the study, starting with the screening visit through 90 days after the last dose of study treatment.

18) Any condition that would, in the investigator's judgment, interfere with full participation in the study, including administration of study drug and attending required study visits; pose a significant risk to the subject; or interfere with interpretation of study data.

5. TREATMENT

5.1. Treatment Assignment

5.1.1. Subject Numbering and Treatment Assignment

All subjects will sign an ICF and be assigned a unique 4-digit subject number within the case report form (CRF). The subject numbering process is defined in the CRF completion guidelines.

Investigative sites must complete all applicable CRFs for subjects consented to the trial, even if the subject is not treated with study drug.

Dose level and cohort assignment will occur at time of subject enrollment and as indicated by the sponsor. Dose level and cohort assignment will be maintained within the CRF per the CRF completion guidelines.

All subjects will be assigned to a cohort to receive NC318; there is no placebo.

NC318 must be administered the same day the investigational product is assigned. If there is a delay in the administration of investigational product such that it will not be administered within the specified timeframe, the sponsor must be notified *immediately*.

Randomization and Blinding

This is an open-label nonrandomized study; therefore, randomization and blinding do not apply.

5.2. Study Drug

5.2.1. Description and Administration

The study drug (NC318) is available in sterile frozen liquid or sterile lyophilized formulations for IV infusion over a minimum of 30 minutes on Day 1 of each cycle (if the infusion is interrupted for any non-clinical reason, sites must notify the medical monitor). The drug product will be provided in 10 ml vials. Each vial will have 400 mg NC318. The concentration of the NC318 frozen liquid formulation is 60mg/mL; total deliverable volume is 6.7 ml. When reconstituted with sterile water, the concentration of the NC318 lyophilized product is 100mg/mL; total deliverable volume is 4.0 mL. For preparation of the study drug for infusion, NC318-01 should be diluted with

normal saline to a concentration no less than 0.2 mg/ml. The infusion site should not be used for blood sampling.

5.2.2. Supply Packaging and Labeling

Study drug is supplied in a tamper evident patient kit box containing frozen liquid drug (400 mg, 60 mg/mL) or refrigerated lyophilized NC318 vials (400 mg). For the frozen liquid formulation, the study drug name (NC318), lot number, concentration, amount of drug in vial, volume, and storage conditions appear on the vial. For the lyophilized formulation, the study drug name (NC318), lot number, amount of drug in vial, and storage conditions appear on the vial. The Pharmacy Manual contains additional information regarding supply, packaging, and labeling of study drug.

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution, and usage of study drug in accordance with the Protocol and any applicable laws and regulations.

5.2.3. Storage

Study drug must be stored in a freezer (-20°C to -50°C) if liquid or a refrigerator (2°C to 8°C) if lyophilized, protected from light, in a secure, controlled-access location. Receipt and dispensing of study drug must be recorded by an authorized person at the study site. Study drug may not be used for any purpose other than that stated in the Protocol. The Pharmacy Manual contains additional information regarding storage of study drug.

5.2.4. Accountability

Detailed information such as requirements for accountability and disposal of study drug can be located within the Pharmacy Manual, which will be provided separately.

5.3. Treatment Compliance

Compliance with study drug dosing will be calculated by the sponsor based on the drug accountability and infusion records documented by the site staff and monitored by the sponsor/designee.

5.4. Treatment Interruptions and Adjustments

5.4.1. Dose Modifications

Selections and modifications to the study drug are planned for dose-escalation cohorts (after consultation with the medical monitor). Dose interruptions and modifications also may occur for individual study subjects. The identification of DLTs will define the doses used in planned cohorts (see Section 5.4.2). The occurrence of DLTs and other toxicities (related or unrelated to study drug) will guide decisions for treatment interruptions and discontinuation for individual subjects. Intrasubject dose escalation is not permitted; however, once the RP2D has been determined, ongoing subjects in Phase 1 may be permitted to dose escalate to the RP2D with medical monitor approval.

5.4.2. Dose-Limiting Toxicity and Determination of Maximum Tolerated Dose

The evaluation period for DLTs will begin on Cycle 1 Day 1 and will continue up to and including Study Day (SD) 28. All DLTs will be assessed by the investigator using National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v5.0. A DLT will be defined as the occurrence of any toxicity in Table 3, **except for** events clearly associated with the underlying disease, disease progression, a concomitant medication, or comorbidity.

Table 3: Definition of Dose-Limiting Toxicity (NCI CTCAE v5.0)

Nonhematologic toxicity
Any ≥ Grade 3 nonhematologic toxicity EXCEPT the following: • Transient (≤ 72 hours) abnormal laboratory values without associated clinically significant signs or symptoms. • An event clearly associated with the underlying disease, disease progression, a concomitant medication, or comorbidity. • Asymptomatic changes in lipid profiles. • Asymptomatic changes in amylase and lipase. • Single or non-fasting elevations in blood glucose **Note**: Any grade ≥3 non-hematologic toxicity should count as DLTs except those that are clearly and incontrovertibly due to disease progression or extraneous causes.
Hematologic toxicity
• Grade 3 thrombocytopenia with clinically significant bleeding (i.e., requires hospitalization, transfusion of blood products, or other urgent medical intervention). • Grade 4 thrombocytopenia. • ≥ Grade 3 febrile neutropenia (absolute neutrophil count < 1.0×10^9/L and fever > 101°F/38.3°C). • Grade 4 neutropenia that does not recover to ≤ Grade 2 in ≤ 3 days after interrupting study drug. • Grade 4 anemia not explained by underlying disease or some other concomitant disorder.
Immune-related toxicity
• ≥ Grade 2 ocular irAEs will be considered a DLT. • Grade 3 irAEs that do not improve to baseline or at least Grade 1 in < 5 days with appropriate care or with corticosteroid therapy will be considered a DLT. • Grade 4 irAEs will be considered a DLT regardless of duration.

General
• Inability to receive the planned number of doses within the 28-day DLT period due to treatment-related toxicity, regardless of grade, will be considered a DLT. • Combined elevations in serum ALT >3 times the upper limit of normal (ULN) and bilirubin >2 ULN in the absence of alkaline phosphatase (ALP) elevation (<2 ULN) • Abnormal liver function tests as indicated by the criteria above will be considered a DLT if no other reason can be found to explain the combination of increased liver markers such as viral hepatitis A, B, or C; preexisting or acute liver disease; or another drug capable of causing the observed injury. • Any death not clearly due to the underlying disease or extraneous causes.
MTD
• In Phase 1 of the study, the MTD will be defined as 1 dose level below that at which ≥ one-third of subjects in a cohort have DLTs. • In Phase 2 of the study, toxicities will continue to be monitored. If the cumulative incidence of DLTs occurs in > 33% of subjects after 6 subjects have been observed for at least 28 days, then further enrollment may be interrupted, and the investigators and sponsor will meet and reassess the MTD. All AEs, regardless of the time of occurrence on study, may be considered in determining the appropriate dose, schedule, and MNTD.
MNTD
• Throughout the treatment period, if > 33% of subjects (minimum of 6 subjects) experience a ≥ Grade 3 toxicity related to study drug after completing ≥ 4 cycles, then dose administration will be stopped, and the MNTD will be determined in conjunction with the investigators and sponsor based on all available safety data.

Abbreviations: ALP = alkaline phosphatase; DLT = dose-limiting toxicity; irAE = immune-related adverse events; MNTD = maximum number of tolerated doses; MTD = maximum tolerated dose; ULN = upper limit of normal.

5.4.3. Management of Dose-Limiting Toxicities or Other Urgent Situations

In all cases, investigators may employ any measures or concomitant medications, after discussion with the medical monitor (whenever possible), necessary to optimally treat the subject.

5.4.4. Follow-Up of Dose-Limiting Toxicities

Any DLT should be followed until it resolves to baseline or appears to have stabilized for a minimum of 4 weeks (e.g., 28 days). During follow-up, subjects should be seen as often as medically indicated to assure safety.

5.4.5. Procedures for Cohort Review and Dose Escalation

Telephone conferences will be scheduled by the sponsor, or sponsor's delegate, with study investigators to review cohort-specific data and overall safety data, to agree on dose escalation, adjudicate individual high-grade AEs as potentially dose-limiting, and guide other major study decisions.

5.4.6. Criteria and Procedures for Dose Interruptions and Adjustments of Study Drug

Treatment with study drug may be delayed allowing for resolution of toxicity. Subjects may resume treatment if no medical condition or other circumstance exists that, in the opinion of the investigator, would make the subject unsuitable for further participation in the study. For toxicities that occur outside of the 28-day DLT period, the treating investigator should contact the medical monitor to discuss the case of any subject whose treatment has been delayed for more than 4 weeks (28 days).

Instructions for dose modifications and interruptions are outlined in Table 4. Individual decisions regarding dose interruptions and reductions should be made using appropriate clinical judgment in consultation with the medical monitor, considering the relatedness of the AE to the study drug and the subject's underlying condition. Adverse events that have a clear alternative explanation or transient (≤ 72 hours) abnormal laboratory values without associated clinically significant signs or symptoms may be exempt from dose-reduction rules. Dose interruptions are permitted in the case of medical/surgical events or logistical reasons not related to study therapy (e.g., elective surgery, unrelated medical events, subject vacation, and/or holidays). Subjects should be placed back on study therapy within 4 weeks (28 days) of the scheduled interruption unless otherwise discussed with the medical monitor. The reason for interruption should be documented in the subject's study record.

Table 4: Rules for Interruption and Restarting of Study Drug

NCI CTCAE v5.0 Grade/Severity	Timing for Restarting Treatment	Dose Level for Restarting Treatment	Treatment Discontinuation
1-2 (mild-moderate)	Continue treatment at the discretion of the investigator.	N/A	N/A
3 (severe)	Toxicity resolves to Grade 0-1.	Reduce by 1 dose level.[a]	Toxicity does not resolve within 4 weeks (28 days) of last dose, except by approval of the medical monitor. OR Second occurrence of previously resolved Grade 3 AE.
4 (life-threatening)	Permanently discontinue.	N/A	Permanently discontinue.

Abbreviation: N/A = not applicable.
Note: Permanently discontinue for any severe or Grade 3 AE that recurs or any life-threatening event.
[a] No more than 2 dose reductions of study drug are permitted . Subjects should be permanently discontinued from study drug if they have AEs requiring more than 2 dose reductions of study drug.

5.4.7. Definition, Procedures, and Supportive Care Guidelines for Immune-Related Adverse Events

NC318 is an immune modulator, and although no significant toxicities were identified in preclinical models, it is possible that irAEs (both nonserious and serious) similar to those described with approved immunotherapies may occur. Adverse events of a potential immunologic etiology or irAEs may be defined as an AE consistent with an immune phenomenon associated with drug exposure ***after all other etiologies have been eliminated***. Immune-related AEs may be expected based on the nature of NC318, its mechanism of action, and based on reported experience with other immunotherapies. Special attention should be paid to AEs that may be suggestive of potential irAEs. An irAE can occur shortly after the first dose or several months after the last dose of treatment. Suspected irAEs should be discussed with the medical monitor when possible. If there is any suspicion of potential cytokine-release syndrome (CRS), subjects should be treated per institutional policy.

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of drug-related AEs with potential immunologic etiology are outlined in Table 5. Detailed supportive care guidelines for specific irAEs can be in found in Brahmer et al. (Brahmer 2018). For each AE, attempts should be made to rule out other causes, including but not limited to metastatic disease or bacterial or viral infection, which might require specific supportive care.

Table 5: Supportive Care Guidelines for Subjects Exhibiting Immune-Related Adverse Events

NCI CTCAE v5.0 Grade/Severity	Supportive Care
Grade 1 (mild)	• Monitor symptoms and provide symptomatic treatment.
Grade 2 (moderate)	• Monitor symptoms and provide symptomatic treatment. • Consider consultation with specialists as necessary. • Consider systemic corticosteroids per institutional standard of care.
Grade 3 - 4 (severe–life-threatening)	• Monitor symptoms and provide symptomatic treatment. • Consider consultation with specialists as necessary. • Administer corticosteroids per institutional standard of care. • More potent immunosuppressive therapies should be considered for events not responding to systemic steroids after discussing with the medical monitor. • Study drug should be permanently discontinued for clinically significant or severe irAEs or for events where steroid course cannot be tapered below 7.5 mg/day prednisone or equivalent to manage symptoms.

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; irAE = immune-related adverse event; NCI = National Cancer Institute.

5.4.8. Management of Infusion Reactions

Table 6 shows treatment guidelines for subjects who experience an infusion reaction associated with administration of study drug. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. As of 29-SEP-2021 data cutoff, there were 4 with infusion reactions (two grade 1 [n=2] and two grade 2 [n=2]) amongst subjects from Phase 1 study (n=49) and 7 had infusion reactions (Grade 1 [n= 1], Grade 2 [n=5], and Grade 3 [n=1]) amongst subjects from Phase 2 study (n=43).

Table 6: Infusion Reaction Treatment Guidelines

NCI CTCAE v5.0 Grade/Severity	Treatment	Premedication at Subsequent Dose Administration
Grade 1: Mild reaction; infusion interruption not indicated; intervention not indicated.	Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None.
Grade 2: Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 hours.	Stop infusion and monitor symptoms. Additional appropriate medical therapy may include but is not limited to the following: • IV fluids • Antihistamines • NSAIDs • Acetaminophen • Narcotics Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate. Otherwise, dose administration will be interrupted until symptoms resolve, and the subject should be premedicated for the next scheduled dose. Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug administration.	Subject may be premedicated 1.5 h (± 30 min) prior to infusion with the following: • Diphenhydramine 50 mg orally (or equivalent dose of antihistamine). • Acetaminophen 500-1000 mg orally (or equivalent dose of antipyretic).

NCI CTCAE v5.0 Grade/Severity	Treatment	Premedication at Subsequent Dose Administration
Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates). OR **Grade 4:** Life-threatening; pressor or ventilatory support indicated.	Stop infusion. Additional appropriate medical therapy may include but is not limited to: • IV fluids • Antihistamines • NSAIDs • Acetaminophen • Narcotics • Oxygen • Pressors • Corticosteroids • Epinephrine Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. Subject is permanently discontinued from further study drug administration.	No subsequent dose.

Abbreviations: CTCAE = Common Terminology Criteria for Adverse Events; NCI = National Cancer Institute.

5.4.9. Criteria for Permanent Discontinuation of Study Drug

The occurrence of an unacceptable toxicity not caused by the underlying malignancy will be presumed to be related to study drug and will require that the study drug be permanently discontinued. Unacceptable toxicity is defined as follows:

- Grade 4 or a life-threatening AE
- ≥ Grade 2 ocular irAE
- Occurrence of an AE that is related to treatment with the study drug that, in the judgment of the investigator or the sponsor's medical monitor, compromises the subject's ability to continue study-specific procedures, or is considered to not be in the subject's best interest.
- Persistent treatment related AE requiring a delay of study drug beyond 4 weeks (28 days)

5.4.10. Treatment After Initial Evidence of Radiologic Disease Progression

Immunotherapeutic agents may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents and may manifest as a clinical response after an initial increase in tumor burden or even the appearance of new lesions. As a result, scans showing progressive disease should be repeated within 4-6 weeks to rule out pseudoprogression at the discretion of the PI.

If radiologic imaging shows disease progression, then subjects have the option to continue treatment while awaiting radiographic confirmation of progression as outlined in Section 7.6.1.

5.5. Withdrawal of Subjects from Study Drug

5.5.1. Withdrawal Criteria

A subject may withdraw consent at any time for any reason or be removed from the trial at the discretion of the investigator should any untoward effect occur. In addition, a subject may be withdrawn by the investigator or the Sponsor if enrollment into the trial is inappropriate, the trial If a subject is withdrawn, then every reasonable effort should be made to determine the reason for withdrawal, and this information should be recorded in the eCRF.

Discontinuation of study treatment does not represent withdrawal from the study. A subject may discontinue from treatment but agree to remain on-study, as long as the subject does not withdraw consent.

Subjects must be withdrawn from the trial for any of the following reasons:

- The subject is lost to follow-up
- Consent is withdrawn for study (does not agree to follow-up); no additional data collection should occur

Subjects must discontinue from the study treatment but can continue to be monitored for any of the following reasons:

- The subject becomes pregnant
- Consent is withdrawn for further treatment (agrees to follow-up)
- Further participation would be injurious to the subject's health or well being
- Unacceptable toxicity (Section 5.4.9). Subjects with unacceptable toxicities must be withdrawn from study drug but will continue to be followed during the safety follow-up visits as specified in Section 6.4
- The study is terminated by the local IRB or regulatory authority
- The study is terminated by the sponsor
- Confirmed radiographic progression of disease per RECIST v1.1. (except if Sponsor approves treatment continuation)
- Investigator decision to withdraw subject
- If, during the course of the study, a subject is found not to have met eligibility criteria, then the medical monitor in collaboration with the investigator, will determine whether the subject should be withdrawn from the study
- If the subject is noncompliant with study procedure or study drug administration in the investigators opinion then the Sponsor should be consulted for instruction on handling the subject.

Subjects who discontinue study treatment but agree to remain on-study should continue to be followed according to the applicable Post-Treatment Follow-up schedule.

5.5.2. Withdrawal Procedures

If the decision is made to permanently discontinue the study drug, the subject will be withdrawn from the study, and the end-of-treatment (EOT) visit should be conducted. Reasonable efforts should be made to have the subject participate in the follow-up period. The date on which the decision was made to discontinue study treatment and the reason for treatment discontinuation will be recorded in the eCRF.

If a subject discontinues study treatment, the site monitor and Sponsor must be notified.

5.6. Concomitant Medications

All concomitant medications and treatments must be recorded in the eCRF. Any prior medication received up to 30 days before the first dose of study drug will be recorded. Upon discontinuation of study treatment, only post-anticancer treatments and concomitant medications and procedures related to adverse events should be recorded. Any addition, deletion, or change in the dose of these medications will also be recorded. Concomitant treatments/procedures that are required to manage a subject's medical condition during the study will also be recorded in the eCRF.

5.6.1. Permitted Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medications will be recorded on the eCRF, including all prescription, over-the-counter, herbal supplements, IV medications and fluids. If changes occur during the study period, documentation of drug regimen, frequency, route, and date may also be included on the eCRF. **Note**: The use of bisphosphonates and denosumab are permitted in this study.

5.6.2. Restricted Medications

Use of systemic glucocorticoids is restricted to prophylaxis for contrast allergies for radiographic procedures, or to modulate symptoms or treat an AE of suspected immunologic etiology. The use of physiologic corticosteroid replacement therapy may be approved after consultation with the medical monitor.

Note: Non-systemic steroids are allowed (e.g., inhaled, intraocular, intranasal, and topical steroids are allowed). A short course of steroids (prednisone or equivalent) ≤ 10 mg/day may be permitted with medical monitor approval.

Acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs; e.g., ibuprofen) may be used. Due to the risk of liver injury with the use of high doses of acetaminophen, subjects should be advised to stay within the recommended daily dose of acetaminophen.

5.6.3. Prohibited Medications

Medications or vaccinations specifically prohibited in the exclusion criteria (see Section 4.2) are not allowed during the ongoing study. If there is a clinical indication for one of these medications or vaccinations specifically prohibited during the study, discontinuation from study drug may be required. The investigator should discuss any questions regarding this with the medical monitor. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician. However, the decision to continue the subject on study drug or vaccination schedule requires the agreement of the investigator, the sponsor, and the subject.

Subjects are prohibited from receiving the following therapies during the screening and treatment periods of this study:

- Any anticancer medications, including chemotherapy or biologic therapy other than the study drug.
- Any immunological-based treatment for any reason from screening through safety follow-up visit.

  Note: Completed adjuvant therapy (e.g., vaccines) with medical monitor approval.

  Note: Inhaled or topical steroids and systemic steroids at doses ≤ 10 mg/day prednisone or equivalents are allowed.

  Note: Immune suppressants are allowed for treatment of immune toxicities as described in Section 5.4.7 and Brahmer et al. (Brahmer 2018).

  Note: Allergy shots may be permitted after consultation with the medical monitor.

- Investigational agents other than the study drug from screening through the follow-up visits.
- Concomitant radiation therapy.

  Note: Radiation therapy to a symptomatic solitary lesion or to the brain may be considered on an exceptional case-by-case basis after consultation with the medical monitor. The subject must have clear measurable disease outside the radiated field. Administration of palliative radiation therapy will be considered clinical progression for the purposes of determining PFS.
- Live vaccines within 30 days before the first dose of study drug *and* while participating in the study. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, Bacillus Calmette–Guérin, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed. However, intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines and are not allowed.
- COVID-19 vaccine within 14 days prior to receiving the first dose of the study drug *and* during the 28-day DLT period. Note: For 2-dose COVID-19 vaccines, subjects must wait at least 14-days after 2nd vaccine dose administration.

Subjects who, in the assessment by the investigator, require the use of any of the aforementioned treatments for clinical management should be removed from the study. Subjects may receive other medications that the investigator deems to be medically necessary. The exclusion criteria describe other medications that are prohibited in this study.

6. SCHEDULE OF STUDY PROCEDURES

Table 7: Schedule of Assessments

Visit Day (Range)	Protocol Section	Screening	Treatment[a]				Post-Treatment		
			All Cycles	Cycles 1 & 5		Every 8-12 weeks	EOT	Disease Status Follow-up[b]	Survival Follow-Up[c]
			Day 1	Day 2	Day 3	Disease Status			
Evaluation/Window		Approx. 30 days prior to C1D1	+3/±3 days after Cycle 1			± 7 days	Upon discontinuation of study treatment	*If EOT for reason other than Progressive Disease*	Every 3 months
Administrative procedures									
Informed Consent	7.1	X							
Eligibility (I/E) criteria	4	X							
Demographics	7.2	X							
Cancer Hx and Diagnosis	7.2	X							
Medical History	7.2	X[d]							
Prior/concomitant medications	7.3	X[e]	X[e]						
Administer NC318	5.2.1		X						
Post-study anticancer therapy	7.4						X	X	X
Survival status	7.4						X	X	X
Clinical procedures/assessments									
Comprehensive PE	7.5.2.1	X[f]							
Targeted physical assessment	7.5.2.2		X[g]				X[g]		
Vital signs and weight	7.5.3	X	X[h]				X		
ECOG performance	7.5.2.1	X	X				X		
12-lead ECG	7.5.4	X[i]							
AE assessment	7.5.1	X[j]	X[j]						
Laboratory assessments (Table 8 and Table 9)	7.5.5	X	X[k]				X		
PK/PD assessments (Table 10 and Table 11)	7.7	X	X[l]	X	X				
Tumor Biopsy	7.8.1	X[m]	X[m]				X[m]		
Efficacy measurements									
Radiologic assessments	7.6.1.1	X[n]				X[o]	X[p]	X	

Abbreviations: C =cycle; D = day; ECG = electrocardiogram; ECOG = Eastern Cooperative Oncology Group; EOT = end-of-treatment; I/E = inclusion/exclusion; PE =physical exam.

a Subjects will begin with the Initial Dosing Schedule of 800mg weekly (Cycle Duration: 7 days (+3 day window after Cycle 1)) and continue with weekly dosing for 8 cycles. Beginning with Cycle 9, subjects will follow the Subsequent Dosing Schedule of 800mg every 2 weeks (Cycle Duration: 14 days (±3 day window). **Note:** Cycle 9 should occur two weeks following the last weekly dose of NC318. Subjects will remain on the Subsequent Dosing Schedule of 800 mg every two weeks until disease progression, withdraw of consent, or intolerable toxicity (whichever comes first). Alternate dosing schedules may also be explored based on emerging data. **For Phase 2 Safety Run-In Only:** The first 4 cycles should be administered every 7 days during the DLT period.

b Subjects who discontinue study therapy for reasons other than disease progression will move to Disease Status Follow-up and will continue to have radiologic imaging performed until 1) withdrawal of consent, 2) documented disease progression, 3) start of new anticancer treatment, 4) death, or 5) the end of the study, whichever occurs first.

c. Subjects that experience site assessed PD or start a new anti-cancer therapy should be followed approximately every 3 months to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first. Updated survival status may be requested by the Sponsor at any time during the course of the study. Survival follow-up may be performed by phone.

d Medical history will include relevant medical or surgical treatment within the last 10 years that are considered to be clinically significant by the investigator.

e Any prior medication received up to 30 days before the first dose of study drug will be recorded. Upon discontinuation of study treatment, only post-anticancer treatments and concomitant medications and procedures related to adverse events should be recorded. Please also refer to Sections 5.6.2 and 5.6.3 for details on prohibited and restricted medications.

f Comprehensive Physical examinations must be performed by a medically qualified individual such as a licensed physician, physician's assistant, or an advanced registered nurse practitioner, as local law permits. The comprehensive physical examination will include assessment(s) of the following organ or body systems: skin; head, eyes, ears, nose, and throat; thyroid; lungs; cardiovascular system; abdomen (liver, spleen); extremities; and lymph nodes, as well as a brief neurological examination. The screening physical examination should also include a measurement of height.

g The targeted physical examination will be a symptom-directed evaluation conducted by the investigator or a medically qualified designee. Notable abnormalities that are considered clinically significant in the judgment of the investigator are to be reported as AEs or Medical History (if applicable at Screening).

h During Cycle 1 subjects will be required to stay at the study site for safety observation for 4 hours post end of infusion. At Cycle 1, vital signs (blood pressure, pulse, respiratory rate, and body temperature) will be assessed pre-dose, at the end of infusion (+10 minutes), and every 60 minutes (± 10 minutes) for 4 hours post end of infusion. On Day 1 of all subsequent cycles subjects will be required to be observed for up 1 hour post end of infusion or per PI discretion, if indicated. Vitals signs will be assessed pre-dose, at the end of infusion (+10 minutes), and thereafter at the PI's discretion. Subjects will also be assessed for the onset of acute AEs.

i A single 12-lead electrocardiogram (ECG) will be performed at screening, using local standard procedures. Clinically significant abnormal findings should be recorded as medical history. Additional ECGs should be performed per PI discretion if any clinically significant abnormal findings are noted or as indicated. All 12-lead ECGs will be performed with the subject in a recumbent or semi-recumbent position after 5 minutes of rest and should generally not be performed within 15 minutes after a blood collection..

j All AEs and SAEs that occur after the consent form is signed but before first study treatment must be reported by the investigator if they are the result of a protocol-specified intervention. From the time of first study treatment through 30 days following end of treatment, all adverse events must be reported by the investigator. SAEs will be collected for 90 days after the end of treatment or 30 days after the end of treatment if the subject initiates new anticancer therapy, whichever is earlier. The severity of AEs will be assessed using NCI CTCAE v5.0 Grades 1 through 4. The NCI CTCAE v5.0 severity of Grade 5 will not be used.

k If a central line is used for study drug infusion, collect blood samples via peripheral blood draw to prevent sample contamination.

l On Day 1 of Cycles 1 and 5, subjects will be required to stay on-site for 4 hours post-dose for collection of PK samples.

m At Screening, archival tumor tissue ≤ 5 years will be acceptable, however a fresh biopsy is preferred. Optional on-treatment tumor biopsy may be performed at Week 7, prior to the first on-study radiologic assessment for subjects who consent at time of informed consent. A second optional biopsy may be performed at EOT for subjects who consent at time of informed consent.

n The initial tumor imaging will be performed within 30 days before the first dose of study drug. Scans performed as part of routine clinical management are acceptable for use as the screening scan if they are of diagnostic quality and performed within 30 days before the first dose of study drug. The same imaging technique should be used for a subject throughout the study. Local reading (investigator assessment with site radiology reading) will be used to determine eligibility and for subject management.

o On-study imaging will be performed at Week 8 and then every 8 weeks (56 days ± 7 days) for the first 6 months and then every 12 weeks (84 days ± 7 days) thereafter. After completing 12 months of treatment, subjects will have imaging performed per standard of care and PI discretion. Imaging should follow calendar days and should NOT be adjusted for delays in cycle starts. If imaging shows disease progression, then another imaging assessment should be performed 4 to 6 weeks later to confirm progression per mRECIST.

p For subjects who discontinue for reasons other than disease progression, radiologic evaluation should be repeated at the time of treatment discontinuation (i.e., date of discontinuation ± 4-week window). If scan was obtained within 4 weeks prior to the date of discontinuation, then a scan at treatment discontinuation (EOT) is not mandatory.

Table 8: Schedule of Local Laboratory Assessments

Visit Day (Range)	Protocol Section	Screening[a]	Treatment	Post-Treatment
			Day 1 of All Cycles	EOT
			D1[b,c]	
Evaluation/Window		**Day - 30 to - 1**	**Predose**	
Comprehensive Metabolic Panel (CMP)[d] – serum	7.5.5	X	X	X
Complete Blood Count (CBC) with differential[e]	7.5.5	X	X	X
Coagulation panel[f]	7.5.5	X		X
Urinalysis[g]	7.5.5	X		X
Endocrine function tests[h]	7.5.5	X	X	X
Hepatitis B and C[i]	7.5.5.2	X		
Serum pregnancy test[j] (childbearing females only)	7.5.5.1	X[j]		X
Urine pregnancy test[k] (childbearing females only)	7.5.5.1		X[l]	

Abbreviations; CBC = complete blood count; CMP = comprehensive metabolic panel; D = day; EOT = end-of-treatment.

a Lab tests must be performed during screening period (within 30 day window).

b Cycle 1 Day 1 laboratory samples must be collected prior to study drug administration. Results should be reviewed by the investigator or qualified designee and found to be acceptable prior to the start of study drug treatment. **Note**: If screening labs were preformed within 7 days of Cycle 1 Day 1, results from screening can be used as indicator to begin study treatment.

c For all subsequent cycles after Cycle 1, pre-dose laboratory procedures may be performed up to 72 hours prior to study drug administration. Results should be reviewed by the investigator or qualified designee and found to be acceptable before the subsequent cycle of treatment is initiated.

d Chemistry required analytes in Table 8.

e Hematology required analytes in Table 8..

f Coagulation panel required analytes in Table 8.

g Urinalysis required analytes in Table 8. Urine dipstick – if positive, perform microscopic urinalysis.

h Endocrine function tests required analytes in Table 8.

i Hepatitis testing required analytes in Table 8.

j Pregnancy testing is required for females of child-bearing potential. A serum pregnancy test must be performed at screening.

k If positive, confirm results with serum pregnancy test.

l For women of child-bearing potential only, Day 1 urine pregnancy must be performed and resulted before study drug administration.

Table 9: Local Laboratory Tests: Required Analytes

Comprehensive Serum Chemistries	Complete Blood Count (w/ differential)	Urinalysis	Hepatitis Screening	Coagulation
Albumin Alkaline phosphatase Alanine aminotransferase Aspartate aminotransferase Bicarbonate Blood urea nitrogen Calcium Chloride Creatinine Glucose Lactate dehydrogenase Phosphorus Potassium Sodium Total bilirubin Direct bilirubin (if total bilirubin is elevated above ULN) Total protein Uric acid Amylase Lipase	Complete blood count, including: Hemoglobin Hematocrit Platelet count Red blood cell count White blood cell count Mean corpuscular volume Differential count, including: Basophils Eosinophils Lymphocytes Monocytes Neutrophils Absolute values must be provided for the following WBC differential laboratory results: White blood cells Lymphocytes Neutrophils Monocytes	Color and appearance pH and specific gravity Bilirubin Glucose Ketones Leukocytes Nitrite Occult blood Protein Urobilinogen	Hepatitis B surface antigen Hepatitis B core antibody HBV-DNA* HCV antibody HCV-RNA* ***Note**: If HCV/HBV antibody or antigen tests are positive, reflex testing (HCV RNA and/or HBV DNA tests) should be performed to confirm results prior to assessing subject eligibility.	PT aPTT INR
		Endocrine Function Tests		**Pregnancy Testing**
		Thyroid-stimulating hormone Free thyroxine Total triiodothyronine (Total T3) or Free triiodothyronine (Free T3)[a]		Female subjects of childbearing potential only - require a serum test at screening and EOT. Urine pregnancy tests should be performed on Day 1 of all other cycles. Pregnancy testing (serum or urine) should be performed as required by local regulations.

Abbreviations: aPTT = activated partial thromboplastin time; DNA = deoxyribonucleic acid; EOT = end-of-treatment; HBV = hepatitis B virus; HCV = hepatitis C virus; HDL = high-density lipoprotein; INR = international normalized ratio; LDL = low-density lipoprotein; PT = prothrombin time; RNA = ribonucleic acid; ULN = upper limit of normal; WBC = white blood cell.
Note: Additional tests may be required, as agreed by investigator and sponsor, based on emerging safety data.
a Based on site standard

Table 10: Schedule of Pharmacokinetic and Anti-Drug Antibody Sampling

Study Visit	Assessment	Timing of Sample Collection
Cycle 1 Day 1	PK and ADA	Pre-infusion
	PK	Post-infusion (+ 10 min) 4 h (± 30 min) post-infusion
Cycle 1 Day 2	PK	24 h (± 60 min) post-infusion
Cycle 1 Day 3	PK	48 h (± 60 min) post-infusion
Cycle 2 Day 1	PK	Pre-infusion
Cycle 3 Day 1	PK and ADA	Pre-infusion
Cycle 5 Day 1	PK and ADA	Pre-infusion
	PK	Post-infusion (+ 10 min) 4 h (± 30 min) post-infusion
Cycle 5 Day 2	PK	24 h (± 60 min) post-infusion
Cycle 5 Day 3	PK	48 h (± 60 min) post-infusion
Cycle 6 Day 1	PK	Pre-infusion
Day 1 of Cycles 9, 13, 17, 21	PK and ADA	Pre-infusion
EOT	ADA	Untimed ADA sample

Abbreviations: ADA = anti-drug antibody; EOT = end-of-treatment; PK = pharmacokinetics.

Table 11: Schedule of Pharmacodynamic (Biomarker) Sampling

Biomarker Assessment	**Study Visit**	**Timing of Sample**		
		Anytime	Pre-Infusion	Post-infusion (+ 10 minutes)
PBMC's	Cycle 1 Day 1		X	
	Cycle 1 Day 2	X		
	Day 1 of Cycles 3, 5, 9, 13, 17, 21		X	
Serum correlative sample for cytokine levels	Cycle 1 Day 1		X	X
	Cycle 1 Day 2	X		
	Cycle 1 Day 3	X		
	Cycle 5 Day 1		X	X
	Cycle 5 Day 2	X		
	Cycle 5 Day 3	X		
	Cycle 9 Day 1		X	X
Plasma ctDNA	Day 1 of Cycle 1		X	
Biopsies	Screening Week 7 (optional) EOT (optional)	X		

Abbreviations: ctDNA = circulating tumor deoxyribonucleic acid; EOT = end-of-treatment; PBMC = peripheral blood mononuclear cells.

6.1. Screening Period

Screening is the interval between signing the ICF and the day the subject is enrolled in the study (e.g., Cycle 1 Day 1).

Screening assessments may be completed over a period of approximately 30 days. Informed consent must be obtained before performing any study-specific procedures that are not considered standard of care. However, procedures conducted as part of the subject's routine clinical management obtained before signing of informed consent may be used for screening or baseline purposes with approval of the medical monitor, provided that the procedure meets the Protocol-defined criteria. Assessments that are required to demonstrate eligibility may be performed over the course of 1 or more days during the screening process.

Results from the screening visit evaluations will be reviewed to confirm subject eligibility before enrollment or administration of study drug. Tests with results that fail eligibility requirements may be repeated **once** during screening if the investigator believes the results to be in error. For screening assessments that are repeated, the most recent available result before enrollment will be used to determine subject eligibility. Additionally, a subject who fails screening may repeat the screening process **1 time** if the investigator believes there has been a change in eligibility status (e.g., after recovery from an infection). Treatment should start as soon as possible after the date of enrollment.

6.2. Treatment Period

The treatment period begins on the day the subject receives the first dose of study drug (Cycle 1 Day 1). Cycle 1 Day 1 should take place within approximately 30 days after the subject has signed the ICF.

Subjects will have regularly scheduled study visits on Day 1 of every cycle. The duration of each cycle is dependent on the dosing schedule:

- **Initial Dosing: 800 mg weekly for 8 Cycles**
    - **Cycle Duration**: 7 days (+3 days after Cycle 1)
- **Subsequent Dosing (Cycle 9 onwards):** 800 mg every 2 weeks
    - **Cycle Duration:** 14 days (±3 days)

For Phase 2 Safety Run-In Only: The first 4 cycles should be administered every 7 days during the DLT period.

Subjects will begin with the Initial Dosing Schedule on Cycle 1 (Day 1) and continue with weekly dosing for 8 Cycles. Beginning with Cycle 9, subjects will follow the Subsequent Dosing Schedule of 800 mg every 2 weeks. **Note**: Cycle 9 should occur two weeks following the last weekly dose of NC318. Subjects will remain on the Subsequent Dosing Schedule of 800 mg every two weeks until disease progression, withdraw of consent, or intolerable toxicity (whichever comes first).

Additional visits will be required on Days 2 and 3 of Cycles 1 and 5. During study visits, the subject will have clinical and laboratory assessments as outlined in Table 7 and Table 8. At certain study visits, subjects will have PK, PD, and biomarker samples obtained (see Table 10 and

Table 11). Toxicities will be monitored continuously and will be graded using the NCI CTCAE v5.0 criteria.

During Cycle 1, subjects will be required to stay at the study site for safety observation for 4 hours post end of infusion. For all other cycles (with the exception of Cycle 5), subjects will be required to stay at study site for 1 hour post end of infusion for observation or per PI discretion if indicated.

It is recommended that the subject does not leave the site for any reason during the safety observation period. During the safety observation period, subjects will be assessed for the onset of acute AEs.

Subjects will continue to receive NC318 until disease progression, withdraw of consent, or intolerable toxicity (whichever comes first). After completing 12 months of treatment, if the subject is deriving benefit from NC318 and has not met any of the protocol defined conditions for withdrawal or discontinuation, the subject will be followed by the PI per standard of care. Subjects approved to continue treatment will remain on dose level assigned at the time of enrollment (unless otherwise approved for dose modification by the medical monitor). Safety reporting will continue through end of treatment to completion of the safety follow-up period.

6.3. End of Treatment Visit

When the subject permanently discontinues (i.e. stops receiving) study drug, the EOT visit should be conducted. If the EOT visit coincides with a regular study visit, then the EOT evaluations will supersede those of that scheduled visit, and the data should be entered in the EOT visit in the eCRF. The subject should be encouraged to return for the follow-up visit(s).

6.4. Post-Treatment Follow-up

6.4.1. Disease Status Follow-up

Subjects who discontinue study drug for a reason **other than** disease progression will move into the disease status follow-up period and should continue to be assessed by radiologic imaging to monitor disease status.

Information regarding post-study anticancer treatment will be collected if new treatment is initiated.

Every effort should be made to collect information regarding disease status until the earliest of the following:

- Withdrawal of consent
- The start of a new anti-cancer therapy
- Documented disease progression
- Pregnancy
- Death
- End of the study

6.4.2. Survival Follow-up

Subjects who experience confirmed disease progression or start a new anticancer therapy will move into Survival Follow-up. Survival Follow-up will occur approximately every 3 months after the end of treatment and will continue until death, withdrawal of consent, or the end of the study, whichever occurs first.

Subjects will be contacted by phone to collect post-study anticancer therapy and survival status. AEs will be collected through 30 days following end of treatment. SAEs will be collected for 90 days after the end of treatment or 30 days after the end of treatment if the subject initiates new anticancer therapy, whichever is earlier.

6.4.2.1. Survival Status

To ensure current and complete survival data is available at the time of database lock, updated survival status may be requested during the study by the Sponsor. Upon Sponsor notification, all subjects who do not/will not have a scheduled study visit or study contact during the sponsor defined time period will be contacted for their survival status (excluding subject that have a previously recorded death event in the eCRF).

6.5. End of Study

The end of the study will occur when all subjects have completed the last study follow-up visit or have discontinued (i.e. stopped receiving) study drug and have completed applicable follow-up assessments. Additionally, subjects will be considered as having completed the study if they meet any of the following criteria:

- Subject dies and a date of death is available
- Subject is known to have died, however the date of death cannot be determined
- Consent is withdrawn for any further contact to this study (Every reasonable effort should be made to determine the reason a subject withdraws prematurely, and this information should be recorded)
- The study is terminated by the sponsor
- The study is terminated by the local IRB or local health authority

6.6. Unscheduled Visits

Unscheduled study visits may occur at any time if medically warranted. Any assessments performed at those visits should be recorded in the eCRF.

7. CONDUCT OF STUDY ASSESSMENTS AND PROCEDURES

Individual study procedures are described in detail below. It may be necessary to perform these procedures at unscheduled timepoints if deemed clinically necessary by the investigator. Furthermore, additional evaluations/testing may be deemed necessary for reasons related to subject safety. In some cases, such evaluation/testing may be potentially sensitive in nature (e.g., HBV, HCV), and thus local regulations may require that additional informed consent be obtained from

the subject. In these cases, such evaluations/testing will be performed in accordance with those regulations. Subjects of childbearing potential must agree to take appropriate measures to avoid pregnancy in order to participate in the study.

7.1. Administration of Informed Consent Form

Valid informed consent must be obtained from the subject before conducting any study-specific procedures using an ICF approved by the local IRB/IEC. The ICF should contain all elements required by ICH E6 and describe the nature, scope, and possible consequences of the study in a manner that the subject can understand. Local and institutional guidelines for ICF content and administration must be followed; the original signed ICF must be retained by the investigator, and a copy of the signed ICF must be provided to the study subject. The informed consent process for each subject must be documented in writing within the subject source documentation.

7.2. Demography and Medical History

7.2.1. Demographics and General Medical History

Demographic data and a complete medical and medication history will be collected at screening by the investigator or qualified designee and will include date of birth, race, ethnicity, smoking history, medical and surgical history, and current illnesses. Medical history will include relevant medical or surgical treatment within the last 10 years that are considered to be clinically significant by the investigator.

7.2.2. Disease Characteristics and Treatment History

A disease-targeted medical and medication history will be collected at screening. Details regarding the subject's malignancy under study including date of diagnosis, initial and current cancer stage, primary tumor histology, and prior treatments including systemic, radiation, and surgical procedures will be recorded.

7.3. Prior and Concomitant Medications and Procedures

Prior and concomitant medications and procedures will be reviewed to determine subject eligibility. All concomitant medications and measures must be recorded in the eCRF, and any medication received, or procedure performed within 30 days before the first dose of study drug and up to the end of the follow-up Phase of the study will be recorded in the eCRF. The medication record will be maintained after signing the ICF to document concomitant medications, including any changes to the dose or regimen. Concomitant medications include any prescription, over-the-counter, or natural/herbal preparations taken or administered during the study period. Concomitant treatments and/or procedures that are required to manage a subject's medical condition during the study will also be recorded in the eCRF. Use of GnRH agonists or antagonists and COVID-19 vaccines should also be recorded in the eCRF.

7.4. Poststudy Anticancer Therapy

The investigator or qualified designee will review all new anticancer therapy initiated after the last dose of study drug, including but not limited to systemic therapy, radiotherapy, and surgery.

7.5. Safety Assessments

7.5.1. Adverse Events

Adverse events monitoring will begin at the time the subject signs the ICF. All AEs and SAEs that occur after the consent form is signed but before first study treatment must be reported by the investigator if they are the result of a protocol-specified intervention.

From the time of first study treatment through 30 days following end of treatment, all adverse events must be reported by the investigator. SAEs will be collected for 90 days after the end of treatment or 30 days after the end of treatment if the subject initiates new anticancer therapy, whichever is earlier.

Subjects will be instructed to report all AEs during the study and will be assessed for the occurrence of AEs throughout the study. In order to avoid bias in eliciting AEs, subjects will be asked general, nonleading questions such as "How are you feeling?" All AEs (serious and nonserious) must be recorded on the source documents and eCRFs regardless of the assumption of a causal relationship with the study drug. The definition, reporting, and recording requirementsfor AEs are described in Section 8.1.

7.5.2. Physical Examinations

7.5.2.1. Comprehensive Physical Examination

Physical examinations must be performed by a medically qualified individual such as a licensed physician, physician's assistant, or an advanced registered nurse practitioner, as local law permits. ECOG performance status should be assessed.

The comprehensive physical examination will include assessment(s) of the following organ or body systems: skin; head, eyes, ears, nose, and throat; thyroid; lungs; cardiovascular system; abdomen (liver, spleen); extremities; and lymph nodes, as well as a brief neurological examination. The screening physical examination should also include a measurement of height. Clinically notable abnormalities that are considered clinically significant in the judgment of the investigator are to be reported as AEs or medical history.

7.5.2.2. Targeted Physical Examination

The targeted physical examination will be a symptom-directed evaluation conducted by the investigator or a medically qualified designee. The targeted physical examination will include assessment(s) of the body systems or organs, as indicated by subject symptoms, AEs, or other findings. Clinically notable abnormalities that are considered clinically significant in the judgment of the investigator are to be reported as AEs.

7.5.2.3. Vital Signs and Weight

Vital sign measurements include blood pressure, pulse, respiratory rate, and body temperature. Blood pressure and pulse will be taken with the subject in the recumbent, semi recumbent, or sitting position after 5 minutes of rest.

During Cycle 1 Day 1, vital signs (blood pressure, pulse, respiratory rate, and body temperature) will be assessed pre-dose, at the end of infusion (+10 minutes) and every 60 minutes (± 10 minutes) for 4 hours post end of infusion.

On Day 1 of all subsequent cycles, vitals signs will be assessed pre-dose and at the end of infusion (+10 minutes) and thereafter at the PI's discretion.

It is recommended that the subject does not leave the site for any reason during the safety observation period. Subjects will also be assessed for the onset of acute AEs.

Note: Weight will also be assessed at each study visit. Clinically notable abnormalities that are considered clinically significant in the judgment of the investigator are to be reported as AEs.

7.5.3. Electrocardiograms

All 12-lead ECGs will be performed with the subject in a recumbent or semi-recumbent position after 5 minutes of rest and should generally not be performed within 15 minutes of a blood collection.

A single 12-lead ECG should be performed at screening using local standard procedures. Clinical significant abnormal findings should be recorded as medical history. Additional ECGs should be performed per PI discretion if any clinically significant abnormal findings are noted or as indicated.

The 12-lead ECGs readings will be interpreted by the investigator, or qualified designee at the site to be used for immediate subject management. The decision to include or exclude a subject or withdraw a subject from the study based on a locally analyzed ECG flagged as "Abnormal, Clinically Significant" is the responsibility of the investigator, in consultation with the medical monitor, as appropriate. Clinically significant abnormal findings before signing consent should be recorded as medical history. Clinically significant abnormal findings after signing consent should be recorded as an AE.

7.5.4. Laboratory Assessments

A laboratory local to the study site and subject will perform all clinical laboratory assessments for safety (i.e., blood chemistries, complete blood count (w/ differential), coagulation tests, endocrine function, and urinalysis). The investigative site will enter the laboratory results and laboratory normal ranges into the eCRF. All local laboratory assessments should be performed using standard procedures on the days indicated in Table 7 and Table 8. Table 9 lists the specific laboratory analytes required for each test. Additional testing may be required by the sponsor based on emerging safety data. Additional tests may also be performed if clinically indicated.

Safety Lab tests must be performed during screening period (within 30-day window).

Cycle 1 Day 1 laboratory samples must be collected prior to study drug administration. Results should be reviewed by the investigator or qualified designee and found to be acceptable prior to the start of study drug treatment. **Note**: If screening labs were preformed within 7 days of Cycle 1 Day 1, results from screening can be used as indicator to begin study treatment.

For all subsequent cycles after Cycle 1, pre-dose laboratory procedures may be performed up to 72 hours prior to study drug administration. Results should be reviewed by the investigator or qualified designee and found to be acceptable before treatment is initiated.

7.5.4.1. Pregnancy Testing

A local laboratory serum pregnancy test will be required for all women of childbearing potential during screening and at the EOT visit. Urine pregnancy tests will be performed locally on Day 1 of each cycle as outlined in Table 7, as medically indicated, or per country-specific requirement. Urine pregnancy tests on Day 1 of each Cycle must be performed and resulted before study drug administration. If a urine pregnancy test is positive, then the results should be confirmed with a serum pregnancy test.

If the serum pregnancy test is negative after a urine test was positive, then the investigator will assess the potential benefit/risk to the subject and determine whether it is in the subject's best interest to resume study drug and continue participation in the study.

7.5.4.2. Hepatitis Screening Tests

Hepatitis screening assessments will be performed during the screening period (Table 8) to rule out hepatitis infection; required analytes are shown in Table 9. Generally, hepatitis tests should be performed early in the screening process due to the length of time needed to obtain the results. Additional tests may be performed if clinically indicated.

7.6. Efficacy Assessments

7.6.1. Modified RECIST v1.1 Assessment of Disease

Modified RECIST (mRECIST) will be applied by the site as the primary measure for assessment of tumor response and as a basis for Protocol guidelines related to disease status (e.g., discontinuation of study therapy). As noted in Section 1.3.4, RECIST v1.1 has been adapted to account for the unique tumor responses seen with immunotherapy (Wolchok 2009).

If radiologic imaging shows progressive disease, then tumor assessments should be repeated at a minimum of 4 weeks, but no later than 6 weeks later to confirm progression, with the option of continuing treatment while awaiting radiologic confirmation of progression. Table 12 provides instructions on how to proceed with treatment based on the subject's clinical status once the initial scan showing radiologic evidence of progression is observed.

- Subjects may receive treatment while waiting for confirmation of progression if they are clinically stable as defined by the following criteria:
- Absence of clinically significant signs and symptoms (including worsening of laboratory findings) consistent with disease progression
- No decline in ECOG performance status
- Absence of rapid progression of disease
- Absence of progressive tumor at critical anatomic sites (such as spinal cord compression) requiring urgent alternative medical intervention

Table 12: Imaging and Treatment After First Radiographic Evidence of Progressive Disease

	Clinically Stable		Clinically Unstable	
	Tumor Imaging	**Treatment**	**Tumor Imaging**	**Treatment**
First radiologic evidence of progression	Repeat imaging 4-6 weeks to confirm progression	May continue study treatment at the investigator's discretion while awaiting confirmatory tumor imaging	Repeat tumor imaging 4-6 weeks to confirm progression per physician discretion only	Discontinue treatment
Repeat scan confirms progression	No additional tumor imaging required	Discontinue treatment	No additional tumor imaging required	N/A
Repeat scan shows stable disease, PR, or CR	Continue regularly scheduled tumor imaging assessments	Continue study treatment at the investigator's discretion	Continue regularly scheduled tumor imaging assessments	May restart study treatment if condition has improved and/or clinically stable per investigator's discretion

Abbreviation: N/A = not applicable.

As noted above, if disease progression is observed, then the study site may elect to continue treatment, repeat imaging at a minimum of 4 weeks, but no later than 6 weeks later, and assess tumor response or confirmed progression per mRECIST.

In determining whether or not the tumor burden has increased or decreased, study site investigators should consider all target lesions as well as nontarget lesions. Subjects that are deemed clinically unstable are not required to have repeat tumor imaging for confirmation. If radiologic progression is confirmed by subsequent scan, then the subject should be discontinued from study treatment. If radiologic progression is not confirmed, then the subject should resume or continue studytreatment and have the next tumor imaging according to the protocol schedule (see Table 7). If progression is not confirmed and the subject continues on treatment, then the date of the next scan that documents disease progression (and is confirmed by a second scan at least 4 weeks, but no later than 6 weeks later) will be considered the date of disease progression.

If a subject has confirmed radiographic progression (i.e., 2 scans at least 4 weeks, but no later than 6 weeks apart demonstrating progression) per mRECIST, but the subject is achieving a clinically meaningful benefit, an exception to continue treatment may be considered after consultation with the medical monitor. Clinically stable subjects at the confirmatory scan should also have no further increase in the target lesions, no unequivocal increase in nontarget lesions, and no additional new lesions develop (non-worsening disease progression) to continue study treatment.

7.6.1.1. Tumor Imaging

The same imaging technique should be used for a subject throughout the study. The baseline scan must be a contrast computed tomography (CT) or magnetic resonance imaging (MRI), except in circumstances where there is a contrast allergy or with medical monitor approval. When the CT

component of a positron emission tomography/CT uses higher energy and thinner slices, it may be acceptable with medical monitor approval. Images of the chest and abdomen are required for all subjects.

7.6.1.2. Tumor Imaging During Screening

Initial tumor imaging must be performed within 30 days before the first dose of study drug. The site study team must review pre-study images to confirm that the subject has measurable disease per RECIST v1.1. Tumor lesions that are located in a previously irradiated area or in an area subjected to other locoregional therapy should not be selected as target lesions. If a subject only has lesions in an area previously irradiated or subjected to locoregional therapy, then the subject will be allowed to enroll. Additionally, it is recommended that tumor lesions selected for biopsy **not** be selected as target lesions.

CT or MRI scan of the brain will be performed at screening if there are signs or symptoms suggesting that the subject has disease involvement in the CNS. An MRI of the brain will also be required at screening for all subjects with melanoma.

Scans performed as part of routine clinical management are acceptable for use as the screening scan if they are of diagnostic quality and performed within 30 days before the first dose of study drug.

7.6.1.3. Tumor Imaging During the Study

The first imaging assessment should be performed 8 weeks after the first dose of study drug and then every 8 weeks (56 days ± 7 days) for 6 months and then every 12 weeks (84 days ± 7 days) thereafter until disease progression is determined. Imaging assessments may be done more frequently if clinically indicated. After completing 12 months of treatment, subjects will have imaging performed per standard of care and PI discretion. **Imaging should not be delayed for delays in cycle starts**.

Per mRECIST v1.1, response should be confirmed by a repeat radiographic assessment not less than 4 weeks from the date that the response was first documented. The scan for confirmation of response may be performed at the earliest 4 weeks after the first indication of response or at the next scheduled scan, whichever is clinically indicated.

Imaging should continue to be performed until documented disease progression, the start of new anticancer treatment, withdrawal of consent, death, or the end of the study, whichever occurs first. Disease progression should be confirmed at least 4 weeks, but no later than 6 weeks after the first scan indicating progression in clinically stable subjects. Subjects who have unconfirmed disease progression may continue on treatment until progression is confirmed, provided that they have met the conditions detailed in Section 7.6.1. A central imaging vendor will not be used in this study.

7.6.1.4. Imaging During Follow-Up

If the subject discontinues study drug for reasons other than disease progression, imaging assessments should continue at the Protocol-specified interval until documented disease progression, the start of new anticancer treatment, withdrawal of consent, death, or the end of the study, whichever occurs first.

If scan was obtained within 4 weeks prior to the date of discontinuation, then a scan at treatment discontinuation (EOT) is not mandatory. For subjects who discontinue study therapy without confirmed disease progression, a radiologic evaluation should be repeated at the time of treatment discontinuation (i.e., date of discontinuation ± 4-week window). For subjects who discontinue treatment for reasons other than disease progression, every effort should be made to continue monitoring their disease status until 1) withdrawal of consent 2) the start of new anti-cancer therapy, 3) documented disease progression, 4) death, or 5) the end of the study, whichever occurs first.

7.7. Pharmacokinetic Assessments

Timing of PK and ADA assessments are outlined in Table 10. After the pre-dose PK sample is drawn, subjects will begin the study drug infusion. Predose is defined as within 24 hours before administration of study drug. Adjustments to the timing of blood sampling may be made based on emerging PK data. The exact date and time of each PK blood draw will be recorded in the eCRF. Instructions for sample preparation and shipping will be provided in the Laboratory Manual.

7.8. Biomarker and Correlative Assessments

Tumor, whole blood, and plasma samples will be collected at the visits outlined in Table 11. **If a central line is used for study drug infusion, collect blood samples via peripheral blood draw to prevent sample contamination.** Additional biomarker assessments that might be correlated with safety, response, or resistance to treatment beyond those listed (e.g., monitoring inflammatory or immune markers, measuring specific cell populations, tumor markers, RNA profiles of specific cell populations, or measuring cell surface markers by flow cytometry, Western blot, mass spectroscopy, or immunoassay) may be evaluated at the discretion of the sponsor using excess translational biomarker or PK samples. All analyses will be conducted by NextCure or NextCure's designee. For information regarding handling/shipping of specimens, please refer to the Laboratory Manual for the study.

7.8.1. Tumor Biopsies

7.8.1.1. Tumor Tissue Collection Requirements

Tumor biopsies will be collected as specified below:

- **Screening:** A fresh biopsy or collection of archival tumor tissue (≤ 5 years) from a previous biopsy is required at Screening.
    - An archival baseline biopsy obtained within 5 years for other purposes (i.e., not an NC318-01 procedure) may be utilized
    - Fresh tumor biopsies should be taken from nontarget lesions when possible.
    - If a subject is scheduled to have a tumor biopsy for the purposes of this study and it is subsequently determined that tumor tissue cannot safely be obtained, then the subject may still enroll in the study.
- **On-treatment (optional):** An on-treatment biopsy may be obtained anytime duringWeek 7 (before the first radiographic assessment) for subjects who consent for on-treatment biopsy at time of informed consent.

- On-treatment biopsies should be performed at the same site as the screening biopsy whenever possible.

- **At the End of Treatment (optional):** An End of Treatment (EOT) biopsy may be obtained at the time that study treatment has been discontinued for subjects who consent for EOT biopsy at time of informed consent.

Details and methods for obtaining, processing, and shipping the fresh tumor biopsy samples will be provided in the Biopsy Collection Manual for the study.

7.8.1.2. Tumor Tissue Assessment

Tumor biopsy samples will be used to investigate molecular signatures associated with response or resistance to treatment with the study drug. DNA and/or RNA may be extracted from these samples to perform somatic mutation analysis, epigenetic analysis, whole exome sequencing, and genetic expression analysis. Tissue may also be examined by histology and immunohistochemistry or by exploratory methods to evaluate markers of inflammation and T-effector cell populations, growth, signaling, apoptosis, etc. that may be associated with safety, response, or resistance to treatment with the study drug.

7.8.2. Whole Blood Correlative Assessment

Whole blood samples will be used for immune cell population profiling (which may include T lymphocytes, B lymphocytes, myeloid, and natural killer cells). Other assays relevant to the objectives of the study, such as flow cytometry analysis of intracellular cytokines may be performed based upon emerging data.

7.8.3. Plasma Pharmacodynamic Assessment

Plasma samples will be evaluated to assess evidence of NC318 activity.

Plasma samples will be used to examine markers of inflammation or immune modulation (which may include, but are not limited to: IFNγ, IL-2, IL-4, IL-6, and TNFα) pre- and on-treatment with NC318. Other assays relevant to the objectives of the study may be performed based on emerging data.

7.8.4. Timing for Plasma and Whole Blood Assessments

Biomarkers will be analyzed in plasma and whole blood samples collected at baseline and on the days and times outlined in Table 11. Timing for biomarker samples is outlined in Table 11. Throughout the study, the exact date and time of the biomarker blood draws will be recorded in the eCRF along with the date and time study drug was administered in the clinic. For information regarding handling/shipping of specimens, please refer to the Laboratory Manual for the study.

7.9. Other Study Procedures

Not applicable.

8. SAFETY MONITORING AND REPORTING

8.1. Adverse Events

8.1.1. Definitions

For the purposes of this Protocol, an AE is defined as any untoward medical occurrence associated with the use of a drug in humans, whether considered drug related, that occurs after a subject provides informed consent and through the follow-up period.

Both nonserious and serious AEs (SAEs) will be monitored throughout the study.

A pre-treatment event (PTE) is one that occurs after the time of informed consent (e.g. during the screening process) but prior to study drug administration. PTEs directly related to a study test or procedure should be reported during the Screening period. Conditions that were already present at the time of informed consent should be recorded on the Medical History form in the eCRF.

A treatment-emergent adverse event (TEAE) is one that occurs from the time of the first dose of study drug through 90 days after the last dose of study drug. TEAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

Abnormal laboratory values or test results occurring after informed consent constitute AEs only if they induce clinical signs or symptoms, are considered clinically meaningful, require therapy (e.g., hematologic abnormality that requires transfusion), or require changes in the study drug(s).

8.1.2. Reporting

Adverse events monitoring will begin at the time the subject signs the ICF. All AEs and SAEs that occur after the consent form is signed but before first study treatment must be reported by the investigator **if** they are the result of a protocol-specified intervention.

From the time of first study treatment through 30 days following end of treatment, all adverse events must be reported by the investigator. SAEs will be collected for 90 days after the end of treatment or 30 days after the end of treatment if the subject initiates a new anticancer therapy.

AEs (including laboratory abnormalities that constitute AEs) should be described using a diagnosis whenever possible rather than by individual underlying signs and symptoms.

The severity of AEs will be assessed using NCI CTCAE v5.0 Grades 1 through 4. **The NCI CTCAE v5.0 severity of Grade 5 will not be used;** AEs resulting in death will be graded accordingly using Grades 1 through 4 and have the outcome noted as fatal.

If an event is not classified by NCI CTCAE v5.0, the severity of the AE will be graded according to Table 13 to estimate the grade of severity.

Table 13: NCI CTCAE v5.0 Grading Scale

Grade	Clinical Characteristics
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate activities of daily living.
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.
Grade 4	Life-threatening consequences: urgent intervention indicated.

The occurrence of AEs should be sought by nondirective questioning of the subject during the screening process after signing the ICF and at each visit during the study. Adverse events may also be detected when they are volunteered by the subject during the screening process or between visits, or through physical examination, laboratory test, or other assessments. To the extent possible, each AE should be evaluated to determine:

- The severity grade (NCI CTCAE Grade 1 to 4).
- Whether there is at least a reasonable possibility that the AE is related to the study treatment: suspected (yes) or not suspected (no).
- The start and end dates, unless unresolved at final follow-up.
- The action taken with regard to study drug.
- The event outcome (e.g., not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequelae, fatal, unknown).
- The seriousness, as per SAE definition provided in Section 8.3.1

Unlike routine safety assessments, SAEs are monitored continuously and have special reporting requirements (see Section 8.3.2).

All AEs should be treated appropriately. If an AE is treated with a concomitant medication or nondrug therapy, this action should be recorded on the AE form in the eCRF and the treatment should be specified on the Prior/Concomitant Medications or Procedures and Non-Drug Therapy form in the eCRF.

Once an AE is detected, it should be followed until it has resolved or until it is judged to be permanent; assessment should be made at each visit (or more frequently if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat the event, and the outcome.

When the severity of an AE changes over time for a reporting period (e.g., between visits), each change in severity will be reported as a separate AE until the event resolves. For example, 2 separate AEs will be reported if a subject has Grade 1 diarrhea, meeting the definition of an AE, that lasts for 3 days before worsening to a Grade 3 severity. The Grade 1 event will be reported as an AE with a start date equal to the day the event met the Grade 1 AE definition and a stop date equal to the day that the event increased in severity from Grade 1 to Grade 3. The Grade 3 event

will also be reported as an AE, with the start date equal to the day the event changed in intensity from Grade 1 to Grade 3 and a stop date equal to the day that the event either changed severity again or resolved.

8.2. Laboratory Test Abnormalities

Laboratory abnormalities that constitute an AE in their own right (considered clinically meaningful, induce clinical signs or symptoms, require concomitant therapy, or require changes in study drug) should be recorded on the AE form in the eCRF. Whenever possible, a diagnosis rather than a symptom should be provided (e.g., "anemia" instead of "low hemoglobin"). Laboratory abnormalities that meet the criteria for AEs should be followed until they have returned to normal or an adequate explanation of the abnormality is found. When an abnormal laboratory test result corresponds to a sign or symptom of a previously reported AE, it is not necessary to separately record the laboratory test result as an additional event.

Laboratory abnormalities that do not meet the definition of an AE should not be reported as AEs. A Grade 3 or 4 (severe) AE does not automatically indicate an SAE unless it meets the definition of serious, as defined in Section 8.3.1. A dose modification for the laboratory abnormality may be required (see Section 5.4.6) and should not contribute to the designation of a laboratory test abnormality as an SAE.

8.3. Serious Adverse Events

8.3.1. Definitions

A serious adverse event (SAE) is defined as an event that meets at least 1 of the following criteria:

- Is fatal or life-threatening.
- Requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is a result of:
    - A routine treatment or monitoring of the studied indication not associated with any deterioration in condition.
    - An elective surgery or preplanned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the ICF.
    - A treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE and not resulting in hospital admission.
    - Any social reasons and respite care, in the absence of any deterioration in the subject's general condition.
- Results in persistent or significant disability, incapacity, or a substantial disruption of a person's ability to conduct normal life functions.
- Constitutes a congenital anomaly or birth defect.
- Is an important medical event or a medically significant event that may not result in death, be immediately life-threatening, or require hospitalization but may be considered serious when, based on appropriate medical judgment, the event may jeopardize the subject or may require medical or surgical intervention to prevent 1 of the outcomes listed above.

Symptoms and clinical manifestations of disease progression can be classified as an SAE (if it meets the above criteria), however, progressive disease is not an SAE.

8.3.2. Reporting

Within 24 hours of identifying an SAE, regardless of the presumed relationship to the investigational product, the investigator or qualified designee must complete the SAE Report Form and submit it to the Sponsor or its designee. Instructions for SAE reporting will be provided by the CRO.

The sponsor is responsible for reporting certain SAEs as expedited safety reports to applicable regulatory authorities, ethics committees, and participating investigators, in accordance with ICH Guidelines and/or local regulatory requirements. The sponsor may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that investigators submit additional information requested by the sponsor or delegate as soon as it becomes available.

Investigators should provide all available information at the time of SAE Report Form completion. Investigators should not wait to collect additional information to fully document the event before notifying Sponsor or its designee of an SAE. When additional information becomes available, investigators should submit a follow-up SAE Report Form (separate from the initial report form) with the new information. Any follow-up information to an SAE also needs to be provided to Sponsor or its designee within 24 hours of learning of the new information.

8.3.3. Reporting of an Overdose

An overdose is defined as a subject receiving a dose of investigational product in excess of that specified in the Investigator's Brochure (IB), unless otherwise specified in this protocol.

Any overdose of a study subject with the investigational product, with or without associated AEs/SAEs, is required to be reported within 24 hours of knowledge of the event to Sponsor or its designee (see Section 8.3.2 for contact information). If the overdose results in an AE, the AE must also be recorded on the AE eCRF. Overdose does not automatically make an AE serious, but if the consequences of the overdose are serious, for example death or hospitalization, the event is serious and must be reported as an SAE. NextCure does not recommend specific treatment for an overdose. The investigator will use clinical judgment to treat any overdose.

8.4. Emergency Unblinding of Treatment Assignment

Not applicable.

8.5. Pregnancy

Pregnancy, in and of itself, is not regarded as an AE unless there is suspicion that study drug may have interfered with the effectiveness of a contraceptive medication or method. When a pregnancy has been confirmed in a subject (or a male subject's partner) during maternal or paternal exposure to study drug, within 90 days of the last dose of study drug or within 30 days after cessation of treatment if the subject initiates new anticancer therapy, the following procedures should be followed to ensure subject safety:

- The study drug must be discontinued immediately (female subjects only).

- Consent must be obtained from female partners of male subjects.
- The investigator must complete and submit the NextCure Clinical Trial Pregnancy form to the sponsor or its designee within 24 hours of learning of the pregnancy.
- A serum pregnancy test must be performed to confirm the urine pregnancy test result (female subjects only).
- If a negative serum test does not confirm the urine pregnancy test result, then:
    - The investigator will use his or her expert judgment, based on an assessment of the potential benefit/risk to the subject, to determine whether it is in the subject's best interest to resume study drug and continue participation in the study.
- The EOT visit evaluations must be performed (female subjects only).

Data on fetal outcome and breastfeeding are collected for regulatory reporting and drug safety evaluation. Follow-up should be conducted for each pregnancy to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications, by following until the first well-baby visit. Pregnancy should be recorded on a Clinical Trial Pregnancy form and reported by the investigator to the sponsor or its designee. Pregnancy follow-up information should be recorded on the same form and should include an assessment of the possible causal relationship to the sponsor's study drug to any pregnancy outcome. The baby should be evaluated for the first 8 weeks or the duration specified in local regulations, whichever is later.

Any SAE occurring during pregnancy must be recorded on the SAE report form and submitted to the sponsor or designee.

8.6. Warnings and Precautions

Special warnings or precautions for the study drug, derived from safety information collected by the sponsor or its designee, are presented in the IB. Additional safety information collected between IB updates will be communicated in the form of Investigator Notifications (INs). Any important new safety information should be discussed with the subject during the study, as necessary, and provided to the IRB. If new significant risks are identified, they will be added to the ICF.

8.7. Data Monitoring Committee

For Phase 1, the sponsor will conduct telephone conferences with investigators in order to review cohort-specific data, to review overall safety data from prior cohorts (if applicable), and to agree on dose escalation, de-escalation, and cohort expansion decisions on a regular basis.

For Phase 2, a formal DMC will be organized and comprised of the following members including but not limited to: a chairperson, two non-participating physicians specializing in oncology, the study medical monitor, a non-voting biostatistician, and representatives of the Sponsor and CRO. The committee will meet on a regular basis to review safety and tolerability throughout the study. Additional information on the DMC will be detailed in the DMC Charter.

8.8. Immune-Related Adverse Events

Adverse events of a potential immunologic etiology or irAEs may be defined as an AE consistent with an immune phenomenon associated with drug exposure after all other etiologies have been eliminated. Immune-related AEs may be expected based on the nature of NC318, its mechanism of action, and reported experience with other immunotherapies. Special attention should be paid to AEs that may be suggestive of potential irAEs. An irAE can occur shortly after the first dose or several months after the last dose of treatment. Guidance for the assessment, diagnosis, and management of irAEs is provided in Section 5.4.7. Suspected irAEs should be discussed with the medical monitor.

Based on the known biology of the S15 pathway and its importance in immune function, as well as clinical experience with other immunotherapies in oncology, immune-related adverse events (irAEs) are the most likely class of toxicity.

8.9. Reporting Product Complaints

Any defects with the investigational products must be reported *immediately* to NextCure by the site with further notification to the site monitor. During the investigation of the product complaint, all investigational products must be stored per instructions in pharmacy manual unless otherwise instructed.

NextCure contact information for reporting product complaints:

Email: ClinicalSupply@nextcure.com
Email subject line should include: “NC318-01 Product Complaint”

Mail: NextCure, Inc.
Attn: Clinical Operations
9000 Virginia Manor Road
Beltsville, MD 20705 USA

9. STATISTICS

This section outlines the statistical analysis strategy and procedures for the study. If, after the study has begun, but prior to any final database lock, changes are made to primary and/or key secondary hypotheses, or the statistical methods related to those hypotheses, then the protocol will be amended (consistent with ICH Guideline E-9). Changes to exploratory or other non-confirmatory analyses made after the protocol has been finalized, but prior to final database lock, will be documented in a supplemental SAP (sSAP) and referenced in the Clinical Study Report (CSR) for the study. Post hoc exploratory analyses will be clearly identified in the CSR.

Tabular summaries will be presented by dose groups. Categorical data will be summarized by the number and percentage of subjects in each category. Continuous variables will be summarized by descriptive statistics including mean, standard deviation, median, minimal, and maximal values. All analyses, unless specified otherwise, will be based on as-treated population, which includes all subjects who receive any dose of NC318. Additional details of statistical analyses will be described in the SAP.

9.1. Study Populations

The full analysis set (FAS) includes all subjects enrolled in the study who received at least one full dose of NC318. This population will be used in all the summaries and analyses except for those of safety analysis set (SAS) and PK. The SAS will include all the subjects who receive any amount of study drug. The summaries and analyses of safety data will be performed on the SAS. The PK analysis set (PAS) will include all the subjects whose blood samples are collected for PK analysis. The PK data summaries and analyses will be performed on the PAS.

9.2. Selection of Sample Size

A maximum of 187 eligible subjects in Phase 1 and Phase 2 will be included in this study.

9.2.1. Sample Size for Phase 1

The primary objective of the dose escalation part in Phase 1 of the study is to determine the MTD or PAD of NC318. A 3 + 3 study design will be used for dose escalation. A maximum of 7 dose levels will be tested. Therefore, a maximum of 42 subjects, who are evaluable for DLTs, will be included in the dose escalation phase. Subjects evaluable for DLTs are defined as the subjects who take a full dose of NC318 and then are followed up for 28 days for assessment of DLTs. Any subjects who don't meet the criteria will be replaced.

Phase 1 of the study may also include safety expansion cohorts (Phase 1b) evaluating doses and schedules equivalent to or lower than the highest dose levels determined to be safe and/or doses determined to be pharmacologically active. Each safety expansion cohort will enroll a total of 9 evaluable subjects (up to 24 additional subjects). If < 3 of 9 evaluable subjects experience a DLT, the cohort will be deemed safe. If more than 1 safety expansion cohort is deemed safe, then the RP2D will be determined in conjunction with the investigators and sponsor based on all available safety, PK, PD, and biomarker results.

Phase 1 enrollment of the study is complete. A total of 49 subjects were enrolled across Phase 1a and Phase 1b. The following cohorts were expanded during Phase 1b: Cohort 2 (80 mg), Cohort 3 (240 mg), and Cohort 4 (400 mg).

9.2.2. Sample Size for Phase 2

Phase 2 of the study will further evaluate the safety, tolerability, preliminary efficacy, PK, and pharmacologic activity of the recommended dose of NC318. In Phase 2, subjects will be stratified after enrollment into cohorts based on S15 expression and tumor type.

9.2.2.1. Cohort 1: Select S15+ Tumors (Lung (adenocarcinoma), HNSCC, and Breast)

This cohort will include subjects who are 1) confirmed as TMB-H and/or MSI-H/dMMR, 2) upon enrollment are confirmed to express S15, and 3) have one of the following tumor types, separated into tumor-specific subgroup: 1a) Lung (adenocarcinoma), 1b) HNSCC, and 1c) Breast cancer. The sample size of each subgroup will be guided by the Simon 2-stage design (Simon 1989). Let 5% be a clinically insignificant response rate for all tumor cohorts. During Stage 1, eachtumor-specific subgroup will enroll 9 evaluable subjects; if no responses are observed within a sub-group, then the sub-group will be discontinued. If 1 or more response is observed within a sub-group, then Stage 2 will begin and 8 additional subjects will be enrolled, for a maximum of

17 subjects within the sub-group. Each tumor-specific sub-group (1a, 1b, and 1c) will be independently assessed.

9.2.2.2. Cohort 2: Alternate Tumors (Other S15+ Tumors/S15- Tumors)

This basket cohort will include all other enrolled subjects who are confirmed as TMB-H and/or MSI-H/dMMR but do not fall under the criteria to be included in Cohort 1. Based on the result of S15 expression, subjects will be stratified after enrollment into two groups within the cohort: 2a) Other S15+ Tumors and 2b) Select 15- Tumors. Each group in the cohort will be guided by the Simon 2-stage design (Simon 1989). Let 5% be a clinically insignificant response rate for all tumor types. During Stage 1, 9 subjects will be enrolled into each group; if no response is observed within a group, then the group will be discontinued. If 1 or more responses is observed within a group, then Stage 2 will begin and 8 additional subjects will be enrolled, for a maximum of 17 subjects per group. Each group (2a and 2b) will be assessed independently.

9.3. Level of Significance

There is no formal hypothesis test planned for this study. A statistically significant level of 0.05 is used for calculation of CI.

9.4. Interim Analysis

No formal interim analysis is planned. However, the safety, PK and efficacy data will be regularly reviewed and monitored for deciding on dose escalation in Phase 1 and continuation in Phase 2 of the study.

9.5. Statistical Analyses

9.5.1. Summary of Baseline Characteristics, Demographics and Other Analyses

The number and percentage of subjects screened, treated, and the primary reason for discontinuation will be displayed. Demographic variables (e.g., age, gender), baseline characteristics, cancer history and diagnosis, and prior and concomitant therapies will be summarized either by descriptive statistics or categorical tables.

9.5.2. Safety Analyses

The safety assessments include adverse events, laboratory tests, and vital signs. All the safety data in the SAS will be summarized and listed by phase, treatment group, and cohort. Count and percentage of AE will be provided.

9.5.2.1. Adverse Events

A treatment-emergent adverse event (TEAE) is any AE either reported for the first time or worsening of a pre-existing event after the first dose of study drug. Analysis of AEs will be limited to TEAEs, but data listings will include all AEs regardless of their timing to study drug administration. Adverse events will be tabulated by the MedDRA preferred term and system organ class. Severity of AEs will be based on the NCI CTCAE v5.0 using Grades 1 through 4.

The subset of AEs considered by the investigator to have a relationship to study drug will be considered to be treatment-related AEs. If the investigator does not specify the relationship of the AE to study drug, then the AE will be considered treatment-related. The incidence, frequency, duration, and severity of all AEs (regardless of causality) will be tabulated.

9.5.3. PK Analyses

The PK analyses will be based off the PAS, and PK time-concentration data will be summarized, listed, and plotted. PK parameters (AUC_{0-336}, $AUC_{0-\infty}$, C_{max}, $t_{½}$, etc.) derived from time-concentration data will be summarized.

9.5.4. Efficacy Analyses

The response will be assessed by CT/MRI image during screening and every 8 weeks (± 7 days) post-dosing for 6 months and then every 12 weeks (± 7 days) thereafter (or per standard of care). The efficacy study endpoints include ORR, DCR, DoR, PFS, and OS. Tumor response will be determined according to RECIST v1.1. Time-to-event data (DoR, PFS, and OS) will be analyzed using the Kaplan-Meier method. All the efficacy data will be summarized by phase, dose group, and cohort.

- ORR includes RECIST v1.1 complete and partial response (CR or PR). ORR with 95% exact CI will be summarized by phase, dose group, and cohort.
- DCR includes RECIST v1.1 complete, partial response, and stable disease (CR, PR or stable disease). DCR with 95% exact CI will be summarized by phase, dose group, and cohort.
- DoR is defined as the duration from the first documented ORR to the first documented progressive disease or death due to any cause, whichever occurs first. For subjects who are alive and progression-free at the time of discontinuation from study, data cutoff for analysis, or use of other anti-cancer drug, DoR will be censored at the last tumor assessment date.

  The DoR will only be analyzed for the subgroup of subjects with an objective response.

  DoR = (progressive disease, death, or censored date) – (date of first response + 1)
- PFS is defined as from starting study treatment to the first documented progressive disease or death due to any cause, whichever occurs first. For subjects who are alive and progression-free at the time of discontinuation from study, data cutoff for analysis, or use of other anti-cancer drug, DOR will be censored at the last tumor assessment date.

  PFS = (progressive disease, death, or censored date) – (date of first dose of study drug + 1)
- OS is defined as from starting study treatment to death due to any cause. For subjects who are alive at the time of discontinuation from study or data cutoff for analysis, OS will be censored at the date of last known alive (LKA).

  OS = (Death or LKA date) – (date of first dose of study drug + 1)

9.5.5. Pharmacodynamic and Biomarkers Analysis

The PD and biomarkers are explorative for this study. These data will be listed only.

Descriptive statistics will be the primary methods for the exploratory analyses. Among the variables to be included in the exploratory analyses are:

- Correlation analysis between PD biomarkers and the disease response to treatment with NC318.

The immunogenic potential of NC318 will be assessed by summarizing the number and percentage of subjects who develop detectable ADAs. The impact of ADAs on PK will be assessed if data allow. Samples will be collected for evaluating neutralizing capacity of ADAs in the future.

9.6. Analyses for Data Monitoring Committee

No formal analysis will be performed for Data Monitoring Committee purposes.

10. STUDY AND DATA MANAGEMENT

10.1. Training of Study Site Personnel

Before the first subject is entered into the study, a NextCure representative will review and discuss the requirements of the Clinical Study Protocol and related documents with the investigational staff and train them in any study-specific procedures and system(s) utilized.

The Principal Investigator (PI) will ensure that appropriate training relevant to the study is given to all of these staff, and that any new information relevant to the performance of this study is forwarded to the staff involved.

The PI will maintain a record of all individuals involved in the study (medical, nursing and other staff).

10.2. Monitoring of the Study

During the study, a NextCure representative will have regular contacts with the study site, including visits to:

- Provide information and support to the investigator(s).
- Confirm that facilities remain acceptable.
- Confirm that the investigational team is adhering to the protocol, that data are being accurately and timely recorded in the eCRFs, that biological samples are handled in accordance with the NC318-01 Laboratory Manual and that study drug accountability checks are being performed.
- Perform source data verification (a comparison of the data in the eCRFs with the subject's medical records at the hospital or practice, and other records relevant to the study) including verification of informed consent of participating subjects. This will require direct access to all original records for each subject (e.g., clinic charts).

- Ensure withdrawal of informed consent to the use of the subject's biological samples is reported and biological samples are identified and disposed of/destroyed accordingly, and the action is documented, and reported to the subject.

The NextCure representative will be available between visits if the investigator(s) or other staff at the center needs information or advice about the study conduct.

10.2.1. Source Data

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Data reported in the eCRF derived from source documents should be consistent with the source documents.

The primary source document for this study will be the subject's medical record. If the investigator(s) maintains separate research records, both the medical record and the research records will be considered the source documents for the purposes of auditing the study.

Data recorded on source documents will be entered into eCRFs. The investigator must promptly review the completed eCRFs for each subject. A study monitor representing the sponsor will review the source documents against the eCRF on a regular basis throughout the study.

The PI at each/the center should comply with all the terms, conditions, and obligations of the Clinical Study Agreement, or equivalent, for this study. In the event of any inconsistency between this Clinical Study Protocol and the Clinical Study Agreement, the terms of Clinical Study Protocol shall prevail with respect to the conduct of the study and the treatment of subjects and in all other respects, not relating to study conduct or treatment of subjects, the terms of the Clinical Study Agreement shall prevail.

Agreements between NextCure and the PI must be in place before any study-related procedures can take place, or subjects are enrolled.

10.2.2. Archiving of Study Documents

The Investigator follows the principles outlined in the Clinical Study Agreement.

10.3. Study Timetable and End of Study

An individual subject will be considered to have completed the study if the subject was followed through the last protocol-specified visit/assessment (including telephone contact), regardless of the number of doses of investigational product that was received.

Subjects will be considered not to have completed the study if consent was withdrawn or the subject was lost to follow-up (see Section 5.5.1).

The end of the study ("study completion") is defined as the date of the last protocol-specified visit/assessment (including telephone contact) for the last subject in the study.

10.4. Data Management

Data management will be performed by Syneos Health, on behalf of NextCure, according to the study specific Data Management Plan.

A Web Based Data Capture (WBDC) system will be used for data collection and query handling. The investigator will ensure that data are recorded on the eCRFs as specified in the study protocol and in accordance with the instructions provided.

The investigator ensures the accuracy, completeness, and timeliness of the data recorded and of the provision of answers to data queries according to the Clinical Study Agreement. The investigator will sign the completed eCRFs. A copy of the completed eCRFs will be archived at the study site.

10.5. Medical Monitor Coverage

Each subject will be provided with contact information for the PI and the site coordinator(s). In an emergent situation, a subject may present to a medical facility where the treating health care provider is not involved with this clinical trial. The treating healthcare provider may require additional information on the study drug, and the subject should provide contact information for the site coordinator(s) or the PI.

The PI will then be required to update the medical monitor.

11. ETHICAL AND REGULATORY REQUIREMENTS

11.1. Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice (GCP), and applicable regulatory requirements.

11.2. Subject Data Protection

The ICF will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.

The anonymity of subjects must be maintained. Subjects will be identified by their initials and an assigned subject number on eCRFs and other documents submitted to the sponsor. Documents that identify the subject beyond initials and subject number will not be submitted to the sponsor (e.g.; the signed ICF) and must be maintained in strict confidence by the investigator, except to the extent necessary to allow auditing by the regulatory authorities, site monitor, or sponsor representatives.

NextCure will not provide individual genotype results to subjects, any insurance company, any employer, their family members, general physician, or any other third party, unless required to do so by law.

Extra precautions are taken to preserve confidentiality and prevent genetic data being linked to the identity of the subject. In exceptional circumstances, however, certain individuals might see both the genetic data and the personal identifiers of a subject. For example, in the case of a medical emergency, a NextCure medical monitor or an investigator might know a subject's identity and also have access to his or her genetic data. Also, Regulatory authorities may require access to the relevant files, though the subject's medical information and the genetic files would remain physically separate.

11.3. Ethics and Regulatory Review

An IRB/IEC should approve the final study protocol, including the final version of the ICF and any other written information and/or materials to be provided to the subjects. The investigator will ensure the distribution of these documents to the applicable IRB/IEC, and to the study site staff.

The opinion of the IRB/IEC should be given in writing. The investigator should submit the written approval to NextCure before enrollment of any subject into the study.

The IRB/IEC should approve all advertising used to recruit subjects for the study.

NextCure should approve any modifications to the ICF that are needed to meet local requirements.

If required by local regulations, the protocol should be re-approved by the IRB/IEC annually.

Before enrollment of any subject into the study, the final study protocol, including the final version of the ICF, is approved by the national regulatory authority or a notification to the national regulatory authority is done, according to local regulations.

NextCure will handle the distribution of any of these documents to the national regulatory authorities.

NextCure will provide Regulatory Authorities, IRB/IEC, and PIs with safety updates/reports according to local requirements, including suspected unexpected serious adverse reactions (SUSARs), where relevant.

Each PI must inform the IRB/IEC of:

- Changes in informed consent
- Revisions of other documents originally submitted for review
- Serious and/or unexpected AEs occurring during the study
- New information that may adversely affect the safety of subjects or the conduct of the study
- Annual update and/or request for re-approval
- Study completion

11.4. Informed Consent

A copy of the proposed ICF must be submitted to the sponsor for review and comment prior to submission to the reviewing IRB/IEC. The ICF must be approved by the IRB/IEC and contain all elements required by national, state, local and institutional regulations or requirements.

The PI at each center will:

- Ensure each subject is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the study
- Ensure each subject is notified that they are free to discontinue from the study at any time
- Ensure that each subject is given the opportunity to ask questions and allowed time to consider the information provided

- Ensure each subject voluntarily provides signed and dated informed consent before conducting any procedure specifically for the study
- Ensure the original, signed ICF(s) is/are stored in the Investigator's Study File
- Ensure a copy of the signed ICF is given to the subject
- Ensure that any incentives for subjects who participate in the study as well as any provisions for subjects harmed as a consequence of study participation are described in the ICF that is approved by an IRB/IEC

11.5. Changes to the Protocol and Informed Consent Form

Protocol revisions will be prepared and approved by NextCure and the medical monitor. Minor revisions will be submitted as administrative changes. If there are any substantial changes to the study protocol, then these changes will be documented in a study protocol amendment and where required in a new version of the study protocol.

All protocol amendments will be signed by the PI and approved by the relevant IRB/IEC and if applicable, also the national regulatory authority approval, before implementation. Local requirements are to be followed for revised protocols. Documentation of IRB/ IEC approval must be forwarded to the sponsor.

NextCure will distribute any subsequent amendments and new versions of the protocol to each PI. For distribution to IRB/IEC see Section 11.3.

If a protocol amendment alters the study design, increases potential risk to the subject or otherwise affects statements in the ICF, the ICF must be revised accordingly and submitted to NextCure and the IRB/IEC for review and approval before the revised ICF is used. The approved ICF must be used to obtain informed consent from new subjects prior to enrollment and must be used to obtain informed consent from subjects already enrolled if they are affected by the amendment.

If local regulations require, any administrative change will be communicated to or approved by each IRB/IEC.

11.6. Audits and Inspections

Authorized representatives of NextCure, a regulatory authority, or an IRB/IEC may perform audits or inspections at the clinical sites, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP, guidelines of the ICH, and any applicable regulatory requirements. The investigator will contact NextCure immediately if contacted by a regulatory agency about an inspection at the site.

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APPENDIX 1. SIGNATURES

Sponsor Signature(s)

A Phase 1/2, Open-Label, Dose-Escalation, Safety and Tolerability Study of NC318 in Subjects with Advanced or Metastatic Solid Tumors

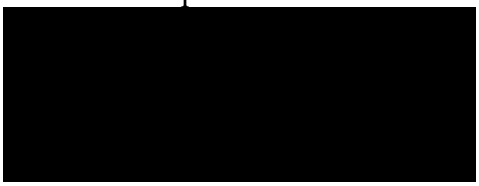
I agree to the terms of this protocol.

[redacted]

Sponsor Signature(s)

A Phase 1/2, Open-Label, Dose-Escalation, Safety and Tolerability Study of NC318 in Subjects with Advanced or Metastatic Solid Tumors

I agree to the terms of this protocol.



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Signature of Principal Investigator

A Phase 1/2, Open-Label, Dose-Escalation, Safety and Tolerability Study of NC318 in Subjects with Advanced or Metastatic Solid Tumors

I, the undersigned, have reviewed this protocol, and I agree to conduct this protocol in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with the International Council for Harmonisation (ICH) guidelines on Good Clinical Practice (GCP), any applicable laws and requirements, and any conditions required by a regulatory authority and/or Institutional Review Board/Independent Ethics Committee (IRB/IEC).

I understand that the protocol may not be modified without written approval of the sponsor. All changes to the protocol must be submitted to the applicable regulatory authority and IRB/IEC and must be approved by the IRB/IEC prior to implementation except when necessary to eliminate immediate hazards to the subjects or when the change(s), as deemed by the sponsor, involves only logistical or administrative changes. Documentation of IRB/IEC approval must be sent to the sponsor immediately upon receipt.

Signature and date: _____

Name and title: _____

Address including postal code: _____

_____

Telephone number: _____

Site/Center Number (if available) _____

APPENDIX 2. ADDITIONAL SAFETY GUIDANCE

Assessment of Severity

Assessment of severity is one of the responsibilities of the investigator in the evaluation of adverse events (AEs) and serious adverse events (SAEs). Severity will be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v5.0 as provided below. The determination of severity for all other events not listed in the NCI CTCAE should be made by the investigator based upon medical judgment and the severity categories of Grade 1 to 4 as defined below.

Grade 1 (mild)	An event that is usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
Grade 2 (moderate)	An event that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the subject.
Grade 3 (severe)	An event that requires intensive therapeutic intervention. The event interrupts usual activities of daily living, or significantly affects the clinical status of the subject.
Grade 4 (life threatening)	An event, and/or its immediate sequelae, that is associated with an imminent risk of death or with physical or mental disabilities that affect or limit the ability of the subject to perform activities of daily living (eating, ambulation, toileting, etc.).

It is important to distinguish between serious criteria and severity of an AE. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 8.3.1. A Grade 3 AE need not necessarily be considered an SAE. For example, a Grade 3 headache that persists for several hours may not meet the regulatory definition of an SAE and would be considered a nonserious event, whereas a Grade 2 seizure resulting in a hospital admission would be considered an SAE.

The NCI CTCAE v5.0 can be downloaded from the Cancer Treatment Evaluation Program (CTEP) (https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf).

Assessment of Relationship

Relationship to Investigational Product

The investigator is required to provide an assessment of relationship of AEs and SAEs to the investigational product.

An event will be considered "not related" to use of the investigational product if any of the following tests are met:

- An unreasonable temporal relationship between administration of the investigational product and the onset of the event (e.g., the event occurred either before, or too long after, administration of the investigational product for it to be considered product-related)
- A causal relationship between the investigational product and the event is biologically implausible (e.g., death as a passenger in an automobile accident)
- A clearly more likely alternative explanation for the event is present (e.g., typical adverse reaction to a concomitant drug and/or typical disease-related event)

Individual AE/SAE reports will be considered "related" to use of the investigational product if the "not related" criteria are not met.

"Related" implies that the event is considered to be "associated with the use of the drug" meaning that there is "a reasonable possibility" that the event may have been caused by the product under investigation (i.e., there are facts, evidence, or arguments to suggest possible causation).

Relationship to Protocol Procedures

The investigator is also required to provide an assessment of relationship of SAEs to protocol procedures on the SAE Report Form. This includes nontreatment-emergent SAEs (i.e., SAEs that occur prior to the administration of investigational product) as well as treatment-emergent SAEs. A protocol-related SAE may occur as a result of a procedure or intervention required during the study (e.g., blood collection, washout of an existing medication). The following guidelines should be used by investigators to assess the relationship of SAEs to the protocol:

Protocol related: The event occurred due to a procedure/intervention that was described in the protocol for which there is no alternative etiology present in the subject's medical record.

Not protocol related: The event is related to an etiology other than the procedure/ interventionthat was described in the protocol (the alternative etiology must be documented in the study subject's medical record).

APPENDIX 3. NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASE AND FOOD AND ALLERGY ANAPHYLAXIS GUIDANCE FOR ANAPHYLAXIS DIAGNOSIS

National Institute of Allergy and Infectious Disease (NIAID) and Food and Allergy Anaphylaxis Network (FAAN) define anaphylaxis as a serious allergic reaction that is rapid in onset and may cause death (Sampson 2006). They recognize 3 categories of anaphylaxis, with criteria designated to capture from 80% of cases (category 1) to >95% of all cases of anaphylaxis (for all 3 categories).

1) Acute onset of an illness (minutes to several hours) with involvement of the skin, mucosal tissue, or both (e.g., generalized hives, pruritus or flushing, swollen lips-tongue-uvula)

   AND AT LEAST ONE OF THE FOLLOWING:

   - Respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, reduced peak expiratory flow (PEF), hypoxemia)
   - Reduced blood pressure or associated symptoms of end-organ dysfunction (e.g., hypotonia [collapse], syncope, incontinence)

2) Two or more of the following that occur rapidly after exposure to a likely allergen for that subject (minutes to several hours):

   - Involvement of the skin-mucosal tissue (e.g., generalized hives, itch-flush, swollen lips-tongue-uvula)
   - Respiratory compromise (e.g., dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia)
   - Reduced blood pressure or associated symptoms (e.g., hypotonia [collapse], syncope, incontinence)
   - Persistent gastrointestinal symptoms (e.g., crampy abdominal pain, vomiting)

3) Reduced blood pressure after exposure to known allergen for that subject (minutes to several hours):

   - Infants and children: low systolic blood pressure (age specific) or greater than 30% decrease in systolic blood pressure
   - Adults: systolic blood pressure of less than 90 mm Hg or greater than 30% decrease from that person’s baseline

APPENDIX 4. EASTERN COOPERATIVE ONCOLOGY GROUP PERFORMANCE STATUS

Grade	Eastern Cooperative Oncology Group (ECOG)
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

Source: Oken 1982.

APPENDIX 5. SUMMARY OF CHANGES

Original Protocol (Version 1.0)	26-JUL-2018
Amendment 1 (Version 2.0)	20-SEP-2018
Amendment 2 (Version 3.0)	17-JUN-2019
Amendment 3 (Version 4.0)	27-AUG-2019
Amendment 3.1 (Version 4.1)	16-MAR-2020
Amendment 4 (Version 5.0)	24-MAY-2021
Amendment 5 (Version 6.0)	18-AUG-2021