TITLE PAGE

Protocol Title:		A First-in-Human Phase 1 Study of NL-201 Monotherapy and in Combination With Pembrolizumab in Patients With Relapsed or Refractory Cancer	
Protocol Number:		NL201-101	
Compound:		NL-201	
Study Phase:		1	
Short Title:		A Phase 1 Study of NL-201 Monotherapy and Combination With Pembrolizumab in Patients With Relapsed or Refractory Cancer	
Sponsor	**Sponsor Name:**	Neoleukin Therapeutics, Inc.	
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Regulatory Agency Identifier Number(s)		IND: 144891 NCT: 04659629	
Protocol Date:		**Document Version**	**Date**
		Original (v 1.0)	29 July 2020
		Amendment 1 (v 2.0)	26 August 2020
		Amendment 1.1 (v 2.1)	04 December 2020
		Amendment 2.0	23 February 2021
		Amendment 3.0	26 August 2021
		Amendment 4.0	03 December 2021
		Amendment 5.0	26 January 2022
		Amendment 6.0	08 September 2022

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SPONSOR SIGNATORY:

NL201-101: A First-in-Human Phase 1 Study of NL-201 Monotherapy and in Combination With Pembrolizumab in Patients With Relapsed or Refractory Cancer

I, the undersigned, have approved Amendment 6.0 of the clinical study protocol with the date of 08 September 2022.

Name and Title	Signature and Date
[redacted]	
[redacted]	

INVESTIGATOR AGREEMENT

NL201-101: A First-in-Human Phase 1 Study of NL-201 Monotherapy and in Combination With Pembrolizumab in Patients With Relapsed or Refractory Cancer

I have read the protocol, including all appendices, and I agree that it contains all the necessary information for me and my staff to conduct this study as described. I will conduct this study as outlined herein, in compliance with current Good Clinical Practice (GCP) standards as defined by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline for GCP, all applicable national, state, and local laws and regulations, and the applicable Institutional Review Board/Independent Ethics Committee (IRB/IEC) and other institutional requirements.

I will provide all study personnel under my supervision copies of the protocol and any amendments, and access to all information provided by Neoleukin or specified designees. I will discuss the material with them to ensure that they are fully informed about NL-201, understand this study, and are able to comply.

_____ _____

Principal Investigator Name (printed) Signature

_____

Date

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
ADA	antidrug antibody
AE	adverse event
AIH	autoimmune hepatitis
ALT	alanine aminotransferase
ANC	absolute neutrophil count
aPTT	partial thromboplastin time
AST	aspartate aminotransferase
AUC	area under the serum concentration time curve
AUC_{inf}	area under the serum concentration time curve from time 0 to infinity
AUC_t	area under the serum concentration time curve from time 0 to time t
BUN	blood urea nitrogen
CAR-T	chimeric antigen receptor T-cell
CD	cluster of differentiation
CFR	Code of Federal Regulation
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CL	clearance
C_{max}	maximum observed serum concentration
CNS	central nervous system
CONSORT	Consolidated Standards of Reporting Trials
CPI	checkpoint inhibitor
CPK	creatine phosphokinase
CR	complete response
CRF	case report form
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTD	cohort target dose
CTFG	Clinical Trial Facilitation Group
CTLA-4	cytotoxic T-lymphocyte-associated protein 4
D	day
DILI	drug-induced liver injury
DLT	dose-limiting toxicity
DMC	Data Monitoring Committee

Abbreviation	Definition
DRESS	drug reaction with eosinophilia and systemic symptoms
DNA	deoxyribonucleic acid
ECG	electrocardiogram
ECHO	echocardiogram
ECI	events of clinical interest
ECOG	Eastern Cooperative Oncology Group
eGFR	estimated glomerular filtration rate
EOI	end of infusion
FDA	Food and Drug Administration
FFPE	formalin-fixed, paraffin embedded
FoxP3	forkhead box P3
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
GLDH	glutamate dehydrogenase
GLP	Good Laboratory Practice
HIV	human immunodeficiency virus
HNSCC	head and neck squamous cell carcinoma
HRT	hormonal replacement therapy
HTID	highest tolerated initial dose
iBOR	immune best overall response
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICI	immune checkpoint inhibitors
iCPD	immune confirmed progressive disease
iCR	immune complete response
ICU	intensive care unit
IEC	Independent Ethics Committee
IFN- γ	interferon gamma
Ig	immunoglobulin
IgG4	immunoglobulin G4
IHC	immunohistochemistry
IL	interleukin

Abbreviation	Definition
INR	international normalized ratio
iPR	immune partial response
irAE	immune-related Adverse Event
IRB	Institutional Review Board
iRECIST	Response Evaluation Criteria in Solid Tumors for use in cancer immunotherapy trials
iSD	immune stable disease
iUPD	immune unconfirmed progressive disease
IV	intravenous
IVF	intravenous fluids
LTFU	long-term follow-up
mAB	monoclonal antibody
MCH	mean corpuscular hemoglobin
MCV	mean corpuscular volume
MRI	magnetic resonance imaging
MedDRA	Medical Dictionary for Drug Regulatory Activities
MTD	maximum tolerated dose
mTPI	modified toxicity probability interval
MUGA	multi-gated acquisition
NE	not evaluable
NK	natural killer
NL	new lesion
NLNT	new lesion-non-target
NLT	new lesion-target
NOAEL	no observed adverse effect level
NSAID	non-steroidal anti-inflammatory drugs
NSCLC	non-small cell lung cancer
ORR	objective response rate
OS	overall survival
PAD	pharmacologically active dose
PBMC	peripheral blood mononuclear cell
PBPK	physiologically-based PK
PD	progressive disease

Abbreviation	Definition
PD-1	programmed cell death 1
PD-L1	programmed cell death ligand 1
PD-L2	programmed cell death ligand 2
PDn	pharmacodynamic(s)
PEG	polyethylene glycol
PFS	progression-free survival
PFTs	pulmonary function tests
PK	pharmacokinetic(s)
[redacted]	[redacted]
PR	partial response
PT	prothrombin time
Q2W	every 2 weeks
Q3W	every 3 weeks
QTc	corrected QT interval
RBC	red blood cell
RCC	renal cell carcinoma
RECIST	Response Evaluation Criteria in Solid Tumors
RFU	radiological follow-up
RNA	ribonucleic acid
RP2D	recommended phase 2 dose
SAE	serious adverse event
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SCR	screening
SD	stable disease
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic-pyruvic transaminase
STAT5	signal transducer and activator of transcription 5
$t_{1/2}$	half-life
TBL	total bilirubin
TMDD	target-mediated drug disposition
TMB	tumor mutational burden
TME	tumor microenvironment
TP	time point
ULN	upper limit of normal

Abbreviation	Definition
Vd	volume of distribution
WBC	white blood cell
WNL	within normal limits
WOCBP	woman of childbearing potential

1 PROTOCOL SUMMARY

1.1 Synopsis

Protocol Title: A First-in-Human Phase 1 Study of NL-201 Monotherapy and in Combination With Pembrolizumab in Patients With Relapsed or Refractory Cancer

Rationale: A majority of patients with relapsed or refractory solid tumors, who have failed available approved lines of therapies, represent a significant unmet medical need. Interleukin (IL)-2 and IL-15 are immune agonists that may elicit antitumor responses in T-cells and natural killer (NK) cells. NL-201 is a novel therapeutic that triggers signaling in both T-cells and NK cells through the IL-2 receptor and may be less toxic than native proteins due to eliminating bias towards off target cells. Among the range of solid cancers that may respond to an immune agonist like NL-201, renal cell carcinoma (RCC) and melanoma cancer are of special interest because recombinant human IL-2 (aldesleukin) has been shown to benefit some patients with these malignancies. Standard of care for malignant melanoma and RCC include immunotherapies with checkpoint inhibitors (CPIs). However, most patients progress or do not tolerate these therapies, and new modalities for the treatment of relapsed or refractory malignant melanoma and RCC are needed.[1] Thus, NL201-101 will evaluate the benefit/risk profile of NL-201 in patients with relapsed or refractory solid tumors.

For the majority of cancer patients, new therapies are urgently needed that can turn cold tumors, tumors that have low numbers T-cells infiltrates, hot, leading to infiltration and activation of immune cells into the tumor microenvironment (TME). In pre-clinical models, NL-201 has been shown to increase T-cell receptor diversity in the TME, and to turn cold tumors hot. The combination of NL-201 with CPIs has shown marked additive and/or synergistic activity in multiple preclinical models. Thus, it is of interest to test this combination in patients as a potential novel regimen.

Objectives and Endpoints

Parts 1 and 2 (NL-201 Monotherapy)

OBJECTIVES	ENDPOINTS
Primary	
• Assess the safety and toxicity profile of NL-201 in patients with advanced solid tumors • Define the recommended phase 2 dose (RP2D) and schedule of NL-201	• Dose-limiting toxicities (DLTs) • Incidence and severity of treatment-emergent adverse events and clinically significant changes in vital signs, electrocardiograms (ECGs), and clinical laboratory tests
Secondary	
• Characterize the pharmacokinetic (PK) profile of NL-201	• PK parameters including but not limited to area under the serum concentration time curve (AUC), maximum observed serum concentration (C_{max}), half-life ($t_{1/2}$), clearance (CL), and volume of distribution (Vd)

OBJECTIVES	ENDPOINTS
• Estimate the immunogenicity of NL-201	• Antidrug antibodies in serum during and after treatment with NL-201
• Estimate the antitumor activity of NL-201 per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 and RECIST for use in cancer immunotherapy trials (iRECIST) criteria	• Best objective response • Objective response rate • Duration of response • Progression-free survival (PFS)
Exploratory	
• Investigate the pharmacodynamic biomarkers of NL-201	• Flow-cytometry analysis of immune cells in blood, eg, for markers of T-cell activation and exhaustion, eg, cluster of differentiation (CD)4, CD8, CD25, forkhead box P3 (FoxP3) • Serum measurements of inflammatory cytokine levels • Analysis of immune characteristics of the tumor microenvironment (eg, multiplex immunohistochemical, transcriptional profiling)
• Estimate additional measures of antitumor activity of NL-201 per RECIST 1.1 and/or iRECIST criteria	• Overall survival (OS) • Clinical benefit rate

Parts 3 and 4 (NL-201 in Combination with Pembrolizumab)

OBJECTIVES	ENDPOINTS
Primary	
• Assess the safety and tolerability of NL-201 in combination with pembrolizumab in patients with advanced solid tumors • Define the RP2D and schedule of NL-201 in combination with pembrolizumab	• DLTs • Incidence and severity of treatment-emergent adverse events and clinically significant changes in vital signs, ECGs, and clinical laboratory tests
Secondary	
• Characterize the PK profile of NL-201 in combination with pembrolizumab	• NL-201 PK parameters including but not limited to AUC, C_{max}, $t_{1/2}$, CL, and Vd
• Estimate the immunogenicity of NL-201 in combination with pembrolizumab	• NL-201 antidrug antibodies in serum during and after treatment with NL-201 in combination with pembrolizumab
• Estimate the antitumor activity of NL-201 in combination with pembrolizumab per RECIST 1.1 and iRECIST criteria	• Best objective response • Objective response rate • Duration of response • PFS
Exploratory	
• Investigate the pharmacodynamic biomarkers of NL-201 in combination with pembrolizumab	• Flow-cytometry analysis of immune cells in blood for modulation of lymphocyte subsets, including markers of T-cell activation and exhaustion, including CD4, CD8, CD25, FoxP3 • Serum measurements of inflammatory cytokine levels • Analysis of immune characteristics of the tumor microenvironment (eg, multiplex immunohistochemical, transcriptional profiling)

OBJECTIVES	ENDPOINTS
• Evaluation of exploratory biomarkers including pharmacodynamic and potential patient selection biomarkers	• Programmed cell death ligand 1 (PD-L1) expression in tumors (as available) • Tumor microenvironment (TME) immune score (as available)
• Estimate additional measures of antitumor activity of NL-201 in combination with pembrolizumab per RECIST 1.1 and/or iRECIST criteria	• OS • Clinical benefit rate

Overall Design:

This is a Phase 1 first-in-human, open-label, dose escalation and cohort expansion study of NL-201 that will be conducted in 4 parts.

Parts 1 and 2 (NL-201 Monotherapy)

Part 1 will be an adaptive modified toxicity probability interval (mTPI) design dose escalation study in adult patients with advanced solid tumors to determine the safety profile and the recommended phase 2 dose (RP2D) and treatment schedule of NL-201 as monotherapy. Part 1 will include intravenous (IV) (Schedule A and B), each with up to approximately 50 patients. In addition, there will be backfill cohorts (up to approximately 12 patients in total) , at certain DMC-cleared dose levels and schedules, to collect, pharmacokinetic, pharmacodynamic and response data in certain tumor types or to explore additional pre-medication regimens (eg, steroids). The decision to enroll patients into backfill cohorts and to define the patients to be enrolled will be made by the Sponsor. Part 1 will enroll up to approximately 115 adult patients in total. Part 2 will be a Simon 2-stage dose expansion cohort study in patients in 2 indications (up to n = 30 per cohort) with exploratory paired biopsies to estimate the tolerability and antitumor activity of NL-201 as monotherapy in these indications.

Parts 3 and 4 (NL-201 in Combination with Pembrolizumab)

Part 3 will be an adaptive mTPI design dose escalation study in up to 42 adult patients with advanced solid tumors to determine the safety profile, RP2D and treatment schedule of NL-201 in combination with pembrolizumab. Part 4 will be a dose expansion phase in 3 cohorts (up to n = 30 per cohort) to confirm the combination RP2D and obtain additional estimation of pharmacokinetics (PK), pharmacodynamics (PDn), and antitumor activity.

All Parts

Patients will enter the screening period, up to 28 days prior to Cycle 1 Day 1 of treatment. PK, PDn, and exploratory tumor biopsy samples will be obtained per the study Schedule of Events (see Section 1.3). Tumor response to treatment will be assessed by radiographic evaluation per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 and RECIST for use in cancer immunotherapy trials (iRECIST) every 6 weeks for 12 weeks, and every 12 weeks thereafter while on study treatment. Patients may continue on treatment until unacceptable toxicity, disease progression per iRECIST with either confirmation or clinical decline, completion of 35 administrations (approximately 2 years) with pembrolizumab (for Parts 3 and 4), withdrawal

of consent, patient or physician decision, study termination, or death. Patients with progressive disease who are considered to be clinically stable and whose progression is considered to be minimal are required to have a confirmatory scan and tumor evaluation after the initial determination of progression. Patients with progressive disease per RECIST criteria may continue on treatment past progression if, in the opinion of the Investigator, the patient is considered to be clinically stable or has experienced clinical benefit from NL-201 alone or in combination with pembrolizumab (see Section 6.1.1.1).

Upon permanent discontinuation of study drug for any reason, an end of treatment visit should be scheduled as soon as possible but within 30 days following cessation of study treatment or before patient initiates new anti-cancer therapy, whichever is earlier. Adverse events (AEs) and concomitant medication will be followed up to 30 days after last dose of NL-201.

For Parts 3 and 4, all AEs will be followed up to 30 days following cessation of study treatment and all SAEs will be followed up to 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anti-cancer therapy, whichever is earlier. Patients who discontinue from treatment due to progressive disease based on RECIST or iRECIST will enter long-term follow-up to assess survival. Patients in Parts 2 and 4 will also need to record any subsequent anti-cancer therapies during long-term follow-up.

For Parts 2 and 4, patients who discontinue from treatment due to reasons other than progressive disease based on RECIST or iRECIST will be followed in radiologic follow-up for documented progressive disease by RECIST 1.1 and will enter long-term follow-up. If subsequent anti-cancer therapies are initiated during the period of time between discontinuation of treatment and progressive disease documentation, the patient will no longer be radiologically assessed for progression and will enter long-term follow-up.

Patients in long-term follow-up will be followed until death, withdrawal of consent, lost to follow-up, or study closure, whichever occurs first, for a maximum of 2 years after the end of treatment.

Part 1: Intrasubject Dose Escalation

Intrasubject dose escalation is permitted with Medical Monitor approval. In cases where no treatment-related Grade 2 or higher adverse events have been observed, dose escalation to a higher dose will be permitted provided that the dose level has been cleared by the DMC.

Part 1: Optional Tumor Biopsy

During dose escalation, paired biopsies are optional. For patients who consent to have paired biopsies, the same lesion will be sampled within 28 days prior to the first dose of NL-201 (ie, pre-treatment biopsy) and on study (ie, post-treatment biopsy). The post-treatment, second biopsy will occur between 5 to 9 days after receiving dose at Cycle 2 Day 1 and for Schedule B patients, before the dosing at Cycle 2 Day 8 visit. The lesion to be biopsied cannot be the only site of measurable disease.

Part 1: IV Dose Escalation Study of NL-201

Dose escalation will follow a mTPI design to guide dose escalation and de-escalation within each schedule. NL-201 will be administered as an IV infusion in 21-day cycles in cohorts of $n \geq 3$ patients at a time. Schedule A cohorts will be dosed every 21 days, while Schedule B cohorts will be dosed on Days 1 and 8 of every 21-day cycle.

The Schedule A and Schedule B cohorts will enroll on a rolling basis in parallel and cohorts will be managed by a Cohort Management Plan. The first new patient of each new dose level shall be observed for 48 hours after their first infusion, prior to enrolling subsequent patients to that dose level and schedule.

In addition, there will be backfill cohorts (up to approximately 12 patients in total) , at certain DMC-cleared dose levels and schedules, to collect, pharmacokinetic, pharmacodynamic and response data in certain tumor types or to explore additional pre-medication regimens (eg. steroids). These patients will not be part of the DLT evaluation but will be included in the safety population.

Refer to Section 6.2.1 for details regarding dose escalation. The final decision in terms of the dose to be applied to the next set of $n \geq 3$ patients will be made by the DMC based upon the mTPI design together with the available safety and laboratory data.

Based on the totality of data arising in Part 1, the DMC will determine a RP2D. If the RP2D requires step-dosing, the step-dosing regimen would be incorporated into dosing for patients in Part 2.

Part 2: Monotherapy Dose Expansion Study of NL-201

This monotherapy dose expansion cohort study will evaluate safety and estimate antitumor activity of NL-201 as monotherapy in diseases that are responsive to IL-2, ie, (1) malignant melanoma and (2) RCC. The RP2D (and treatment schedule) for these cohorts will be determined in Part 1.

Expansion cohorts will individually comprise up to 30 patients each, of which a minimum of 8 patients per cohort will be required to have paired biopsies of the same lesion before and after treatment with NL-201. Pre-treatment biopsies will occur up to 28 days prior to the first dose (Cycle 1 Day 1), while post-treatment biopsies will occur 7 (± 2) days after the Cycle 2 dose.

Part 3: Dose Escalation Study of NL-201 in Combination with Pembrolizumab

Dose escalation will follow a mTPI design to guide dose escalation and de-escalation within each schedule. NL-201 will be administered as an IV infusion in 21-day cycles in cohorts of $n \geq 3$ patients at a time. The starting dose of NL-201 will be at least 1 dose level below the highest dose level that has been recommended as safe and tolerable by DMC from Part 1 of the study.

Schedule A cohorts will be dosed every 21 days, while Schedule B cohorts will be dosed on Days 1 and 8 of every 21-day cycle. A fixed dose of 200 mg pembrolizumab will be administered on Day 1 of every 21-day cycle, regardless of Schedule. Pembrolizumab will be administered before NL-201. NL-201 infusion will start 30-60 minutes after pembrolizumab infusion is complete. Refer to Section 6.1.1 for additional details regarding drug administration. The DMC may modify the NL-201 and pembrolizumab dosing schedule based on emerging safety data.

Schedule A will open first to enrollment. Schedule B can be started subsequently as determined by safety from monotherapy, provided that each dose level must be cleared in Schedule A before enrolling to Schedule B at the same dose level. If Schedule A and Schedule B are enrolling simultaneously, Schedule A enrollment will be prioritized over Schedule B to ensure no delay to study enrollment. The first new patient of each new dose level (for both Schedule A and Schedule B) shall be observed for 96 hours after the conclusion of their infusion(s), prior to enrolling subsequent patients to that dose level and schedule.

The final decision in terms of the dose to be applied to the next set of $n \geq 3$ patients will be made by the DMC based upon the mTPI design together with the available safety and laboratory data.

Based on the totality of data arising in Part 3, the DMC will determine a RP2D for NL-201 in combination with pembrolizumab. If the combination RP2D requires step-dosing, the step-dosing regimen would be incorporated into dosing for patients in Part 4.

Part 4: Dose Expansion Study of NL-201 in Combination with Pembrolizumab

This combination therapy dose expansion cohort will further evaluate safety and estimate antitumor activity of NL-201 in combination with pembrolizumab at the selected RP2D (dose and schedule) determined in Part 3 in diseases that are responsive to IL-2, ie, (1) immune checkpoint inhibitors (ICI) naïve malignant melanoma and (2) ICI naïve RCC, and (3) patients with non-small cell lung cancer (NSCLC), melanoma, or head and neck squamous cell carcinoma (HNSCC) who have received prior treatment with pembrolizumab achieving a best response of stable disease (SD).

Expansion cohorts will individually comprise up to 30 patients each, of which a minimum of 8 patients per cohort will be required to have paired biopsies of the same lesion before and after treatment with NL-201. Pre-treatment biopsies will occur up to 28 days prior to the first dose (Cycle 1 Day 1), while post-treatment biopsies will occur 7 (± 2) days after the Cycle 2 dose.

Number of Patients:

Part 1 will enroll up to approximately 50 patients for each of IV Schedule A and IV Schedule B in cohorts of $n \geq 3$ for dose escalation, and up to approximately 12 patients in total for backfill cohorts at certain dose levels and schedules which have been previously cleared by DMC to collect PK, PD and response data in certain tumor types or to explore additional pre-medication regimens (eg, steroids). In total, up to approximately 115 adult patients will be enrolled in Part 1.

Part 2 will enroll up to 30 patients in each cohort, up to a maximum of 60 patients in total.

Part 3 will enroll up to 21 patients for each of Schedule A and Schedule B in cohorts of n ≥ 3 for a maximum of 42 patients in total. Part 4 will enroll up to 30 patients in each cohort, up to a maximum of 90 patients in total.

Combined enrollment for all parts of the study is up to approximately 310 patients.

Treatment:

Part 1 (NL-201 Monotherapy)

A mTPI design will be used to guide dose escalation and de-escalation within each schedule, with the final decision regarding dose assignment being made by the DMC. In Schedule A, patients will be dosed every 21 days, while in Schedule B patients will be dosed on Days 1 and 8 of every 21-day cycle.

Part 1 (NL-201 IV Monotherapy)

Assessment of toxicity and dose escalation or de-escalation as guided per the mTPI will be made after all DLT-evaluable patients have completed Cycle 1 and the DMC has reviewed the safety data. If dose escalation or dose de-escalation is indicated per mTPI, the DMC will recommend the next dose level. Dose escalation and de-escalation will occur independently for cohorts of each schedule. The proposed starting dose for NL-201 IV will be the lowest pharmacologically active dose (PAD) and is 150- to 300-fold below the C_{max} achieved in monkey.

Monotherapy Cohorts (21-day cycles)		NL-201 dose level (µg/kg)	Maximum dose that may be administered at 1 time (µg)[a]
Schedule A (D1)	Schedule B (D1+D8)		
Planned NL-201 Dose Levels[b]			
1A	1B	0.1	10
2A	2B	0.3	30
3A	3B	1.0	100
4A	4B	3.0	300
5A	5B	6.0	600
6A	6B	12.0	1200
Potential NL-201 Intermediate Dose Levels[b]			
2A-1	2B-1	0.6	60
3A-1	3B-1	1.5	150
3A-2	3B-2	2.0	200
4A-1	4B-1	4.0	400
4A-2	4B-2	5.0	500
5A-1	5B-1	7.5	750
5A-2	5B-2	9.0	900
5A-3	5B-3	10.0	1000

D = day; DMC = Data Monitoring Committee

Monotherapy Cohorts (21-day cycles)		NL-201 dose level (µg/kg)	Maximum dose that may be administered at 1 time (µg)[a]
Schedule A (D1)	Schedule B (D1+D8)		

a Applicable to patients that weigh ≥ 100 kg (dose is capped as to not allow > 100 times the current dose level to be administered to a patient at 1 time).
b At the recommendation of the DMC or based on Sponsor's determination, additional intermediate dose levels may be tested and/or cohorts may be expanded.

The maximum tolerated dose (MTD) is defined as the highest dose for which the probability of a patient experiencing a DLT with NL-201 during Cycle 1 is ≤ 33%. At the discretion of the DMC, and in keeping with the pre-specified mTPI design matrix, additional dose levels may be tested to determine the MTD.

Based on review of DLTs, the DMC may initiate step-dosing to improve the tolerability of NL-201, wherein 1 or more step-doses below the target dose would be provided prior to reaching the target dose (see Section 6.2.1.4). If step-dosing is used, the DLT window would extend from the first dose to 21 days after receiving the target dose (ie, would include the initial reduced-dose cycles in addition to the first target-dose cycle). Once a patient receives the intended target dose for their cohort, subsequent doses for that patient will continue at that dose level unless a delay in dosing of > 6 weeks causes washout of the drug, in which case the step-dosing regimen would be repeated if the patient is to resume dosing.

The DMC will determine the RP2D and treatment schedule (either A or B) for Part 2 by considering the PK, PDn, safety and tolerability, antitumor activity, and the occurrence of any cumulative toxicities over multiple cycles of NL-201.

Part 3 (NL-201 in Combination with Pembrolizumab)

Schedule A: Dosing of pembrolizumab and NL-201 will occur on Day 1 of each 21-day cycle. A fixed dose of 200 mg pembrolizumab will be administered before NL-201. NL-201 infusion will start 30-60 minutes after pembrolizumab infusion is complete.

Schedule B: Dosing of pembrolizumab will occur on Day 1 of each 21-day cycle. Dosing of NL-201 will occur on Day 1 and Day 8 of each 21-day cycle. On Day 1, a fixed dose of 200 mg pembrolizumab will be administered before NL-201. NL-201 infusion will start 30-60 minutes after pembrolizumab infusion is complete.

Combination Cohorts (21-day cycles)			NL-201 dose level (µg/kg)	Maximum dose of NL-201 that may be administered at 1 time (µg)[a]
Schedule A (D1)	Schedule B (D1+D8)	Pembrolizumab (D1)		
Planned NL-201 Dose Level				
P-1A	P-1B	200 mg	0.3	30
P-2A	P-2B	200 mg	1	100
P-3A	P-3B	200 mg	3	300
P-4A	P-4B	200 mg	6	600
P-5A	P-5B	200 mg	12	1200

Potential NL-201 Intermediate Dose Level[b]				
P-2A-1	P-2B-1	200 mg	0.6	60
P-3A-1	P-3B-1	200 mg	1.5	150
P-3A-2	P-3B-2	200 mg	2.0	200
P-4A-1	P-4B-1	200 mg	4.0	400
P-4A-2	P-4B-2	200 mg	5.0	500
P-5A-1	P-5B-1	200 mg	7.5	750
P-5A-2	P-5B-2	200 mg	9.0	900
P-5A-3	P-5B-3	200 mg	10.0	1000

D = day; DMC = Data Monitoring Committee

a Applicable to patients that weigh ≥ 100 kg (dose is capped as to not allow > 100 times the current dose level to be administered to a patient at 1 time)

b At the recommendation of the DMC or based on Sponsor's determination, additional NL-201 intermediate dose levels may be tested and/or cohorts may be expanded.

Parts 2 and 4

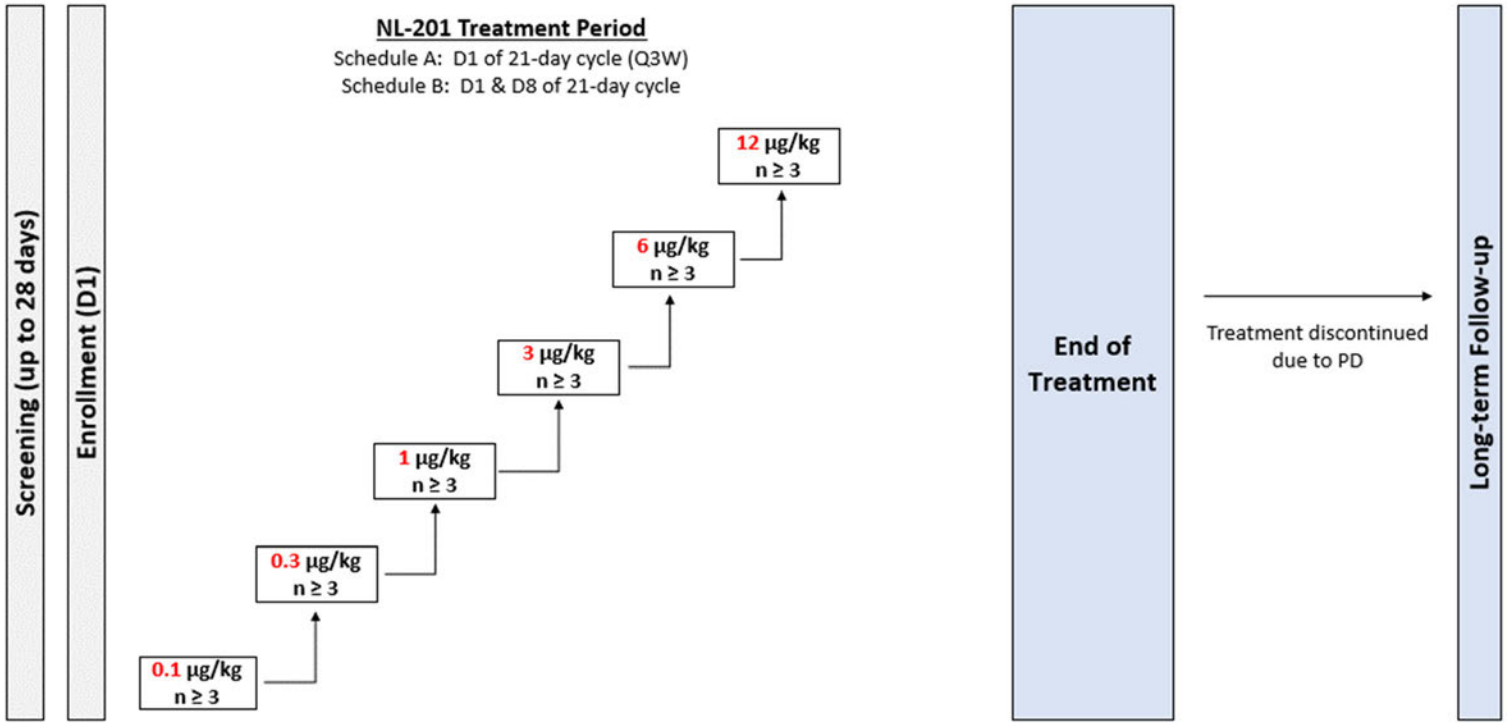
The RP2D and treatment schedule of NL-201 that is determined during dose escalation will be used during dose expansion. No randomization or dose escalation will occur, though dose reduction or delay for toxicity will be allowed.

Data Monitoring Committee: Yes

Disclosure Statement: This is a sequential treatment study with 4 parts that is open-label.

1.2 Schema

Figure 1 Part 1 IV Study Schema[a]



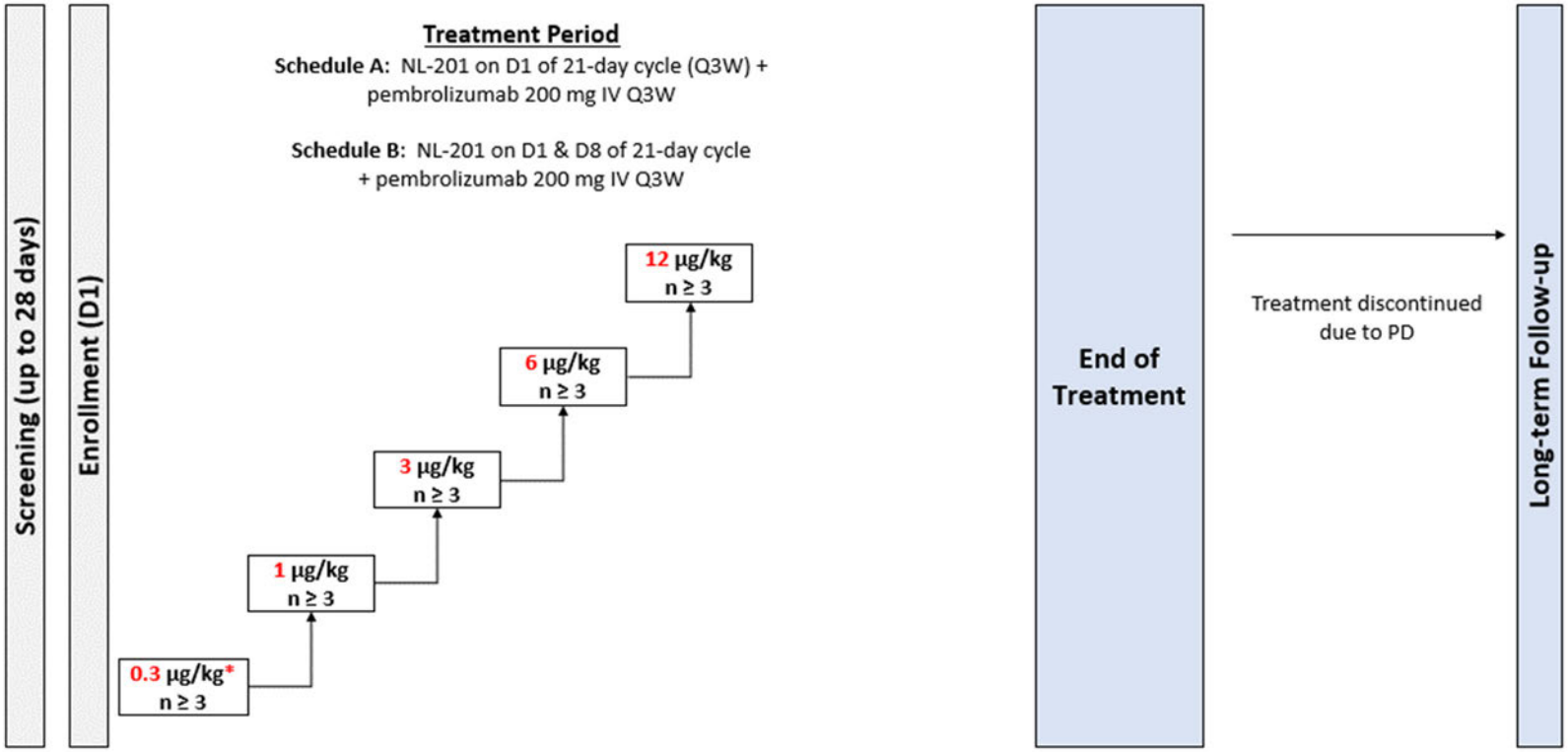
D = day; DMC = Data Monitoring Committee; PD = progressive disease; Q3W = every 3 weeks

a At the recommendation of the DMC or based on Sponsor's determination, additional dose levels may be tested and/or cohorts may be expanded.

Figure 2 Part 2 Study Schema

D = day; PD = progressive disease; RP2D = recommended Phase 2 dose

Figure 3 Part 3 Study Schema (Dose Escalation of NL-201 in Combination with Pembrolizumab)[a]

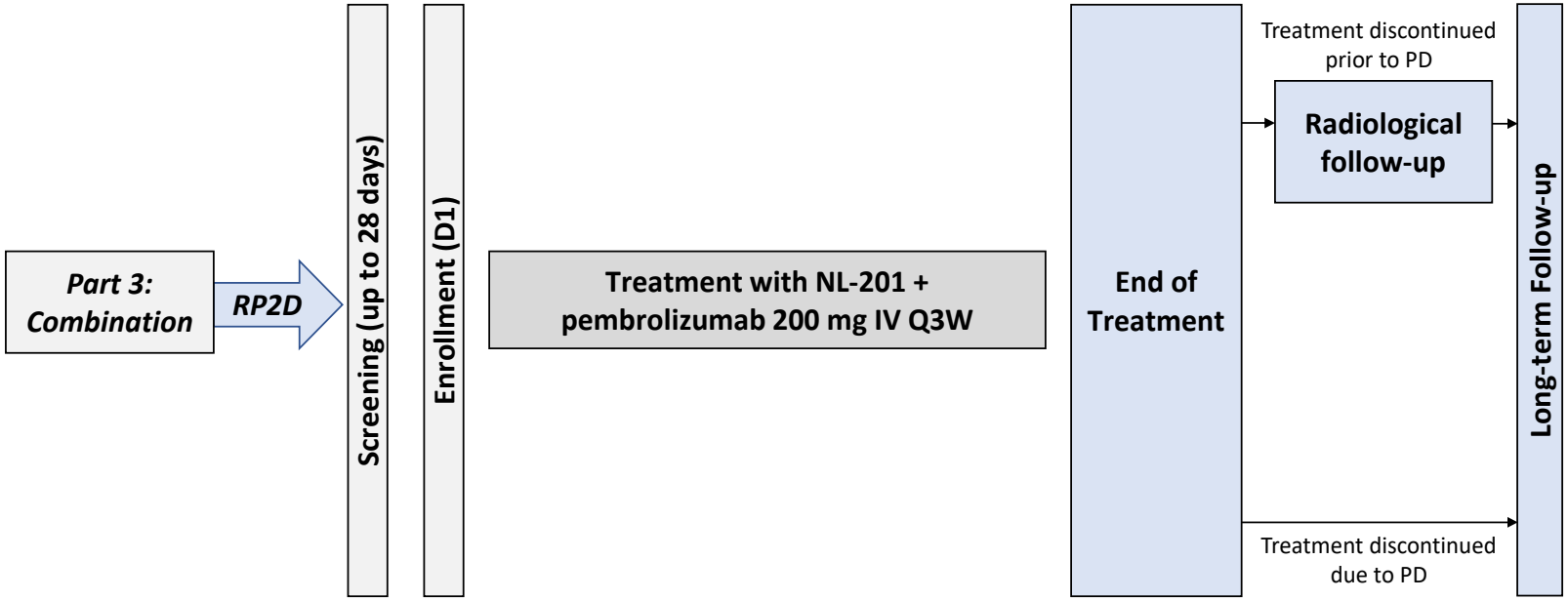


D = day; DMC = Data Monitoring Committee; IV = intravenous; PD = progressive disease; Q3W = every 3 weeks

* The starting dose of NL-201 will be at least 1 dose level below the dose level that has been recommended as safe and tolerable by DMC from Part 1 of the study.

a At the recommendation of the DMC or based on Sponsor's determination, additional dose levels may be tested and/or cohorts may be expanded.

Figure 4 Part 4 Study Schema (Dose Expansion of NL-201 in Combination with Pembrolizumab)



D = day; IV = intravenous; PD = progressive disease; RP2D = recommended Phase 2 dose

1.3 Schedule of Events

SoE Quick Link	Table Title
Table 1	Schedule of Events – Schedule A (Q3W Dosing)
Table 2	Schedule of Events – Schedule B (D1 & D8 Dosing)
Table 3	Schedule of Events – Schedule A (Q3W Dosing) if Step-Dosing is Implemented
Table 4	Schedule of Events – Schedule B (D1 & D8 Dosing) if Step-Dosing is Implemented

Table 1 Schedule of Events – Schedule A (Q3W Dosing)

	SCR[a]	Treatment Period[b] (21-day cycles)									EOT[c]	RFU	LTFU[e]	
		Cycle 1				Cycle 2				Cycle 3+				
		D1	D2	D4	D8	D1	D2	D4	D8	D1				
Treatment Visit Window		0	0	0	±1	±3	0	0	±1	±3				Notes
General and Safety Assessments														
Informed consent	X													
Eligibility criteria	X													
Demography	X													
Medical history	X													
Physical examination	X	(X)			X	X				X	X			Full physical exam at screening and end of treatment; brief physical exam at all other time points (ie, C1D8 and CXD1). (X): Not required if completed within 7 days of Day 1.
Height	X													
Weight	X	X			X	X				X	X			Dose adjustment required if weight changes ≥ 10% from weight used for C1D1 dose (recorded at screening or C1D1 visit or per institutional standard).
Vital signs	X	X	X		X	X				X	X			All D1 assessments include a pre-dose (within 1 h prior to dose) and a post-dose assessment 1h after EOI (± 15 min). Measured in a semi-supine position after 5 min rest.
12-lead ECG	X	(X)				X					X			(X): Completed 6-24 hours after EOI
ECHO	X													If ECHO is not available or appropriate according to the institutional standard, then multi-gated acquisition (MUGA) scan is permitted
PFTs	X													
ECOG performance status	X	(X)				X				X	X			Must be confirmed per Section 8 before dosing C1D1 (X): Assessment can be performed within 48 hours prior to dosing
Hospitalization		X												24-hour hospitalization (may be extended per Investigator discretion). Patients should be advised to stay within a reasonable distance of the clinical site for the first week after the first dose.

	SCR[a]	Treatment Period[b] (21-day cycles)									EOT[c]	RFU	LTFU[e]	
		Cycle 1				Cycle 2				Cycle 3+				
		D1	D2	D4	D8	D1	D2	D4	D8	D1				
Treatment Visit Window		0	0	0	±1	±3	0	0	±1	±3				Notes
AE review	(X)	←								→				(X): Only collect AEs related to study-mandated procedures during screening period. All AEs will be collected during the treatment period and followed for 30 days after the final dose of NL-201. All concomitant medications received within 28 days prior to the first dose of study treatment and up to 30 days after the last dose of study treatment will be recorded. In Parts 3 and 4, SAEs will be collected for 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anti-cancer therapy, whichever is earlier.
Concomitant medication review		←								→				
Survival status													X	
Parts 2 and 4: Subsequent anti-cancer therapy													X	
Local Laboratory Assessments – all samples collected pre-dose unless otherwise indicated														
Pregnancy test	X	(X)				X				X	X			WOCBP only. Must be confirmed per Section 8 before dosing on C1D1. Pregnancy tests may be performed more frequently as required by local regulations. (X): Sample can be collected within 48 hours prior to dosing.
Viral serology	X													
Hematology	X	{X}	X	X	X	X	X	X		X	X			Hematology and safety laboratory tests must be confirmed per Section 8 before dosing on C1D1 {X}: Samples can be collected within 48 hours prior to dosing. [X]: Collected only if biopsy is performed.
Chemistry	X	{X}		X	X	X		X		X	X			
Coagulation	X	{X}			[X]	X			[X]	X	X			
Urinalysis	X	{X}			X	X				X	X			
Parts 3 and 4: Thyroid function tests	X	Every 6 weeks												

	SCR[a]	Treatment Period[b] (21-day cycles)									EOT[c]	RFU	LTFU[e]	
		Cycle 1				Cycle 2				Cycle 3+				
		D1	D2	D4	D8	D1	D2	D4	D8	D1				
Treatment Visit Window		0	0	0	±1	±3	0	0	±1	±3				Notes
Central Laboratory Assessments – all samples collected pre-dose unless otherwise indicated														
Pharmacokinetics		(X)	X	X	X	(X)	X	X		X				All D1 collections include a pre-dose sample (within 1 h prior to dose of NL-201 or pembrolizumab, if applicable) and a post-dose sample 1 h after EOI (± 15 min) of NL-201. D2 and D4 collected 24 h and 72 h after EOI (± 4 h). (X): Additional post-NL-201 dose collections at following time points: • At EOI (+ 10 min) • 2 h after EOI (± 20 min) • 4 h and 8 h after EOI (± 30 min)
Antidrug antibody		X				X				X	X			
Cytokines		(X)	X	X	X	(X)	X	X		X				All D1 collections include a pre-dose sample (within 1 h prior to dose of NL-201 or pembrolizumab, if applicable) and a post-dose sample 1 h after EOI (± 15 min) of NL-201. D2 and D4 collected 24 h and 72 h after EOI (± 4 h). (X): Additional post-dose collection 4 h after EOI (± 30 min)
PDn blood samples		X	X	X	X	X	X	X		X				
Optional Part 1 & 2: Tumor biopsy (for PD analyses)	X								X					Patients who consent to tumor biopsy with readily accessible tumor tissue will have a biopsy performed at screening up to 28 days prior to the first dose (C1D1). Patients will have a 2nd paired biopsy 5 to 9 days after receiving dose at Cycle 2 Day 1 dose (C2D1).
Part 4: Tumor tissue (for PD-L1)	X													Archival biopsy should be within 5 years of screen date for PD-L1 analysis. If archival tissue not available, perform fresh tumor biopsy if readily accessible.
Part 4: Tumor biopsy (for PD analyses)	X								X		(X)			Part 4 patients with readily accessible tumor tissue will have a biopsy performed at screening up to 28 days prior to the first dose (C1D1). Patients will have a 2nd paired biopsy 7 (± 2) days after the Cycle 2 dose. (X): EOT collection is optional.

	SCR[a]	Treatment Period[b] (21-day cycles)									EOT[c]	RFU	LTFU[e]	
		Cycle 1				Cycle 2				Cycle 3+				
		D1	D2	D4	D8	D1	D2	D4	D8	D1				
Treatment Visit Window		0	0	0	±1	±3	0	0	±1	±3				Notes
Efficacy Assessments														
CT scan	(X)	Weeks 1-12: Every 6 weeks (± 3d) Weeks 13+: Every 12 weeks (± 7d)									X[d]	{X}		All scans are calculated from C1D1. RECIST 1.1 and iRECIST assessments continue until disease progression (even if study treatment is discontinued). MRI required only to confirm brain metastasis, if applicable. (X): Completed within 28 days of C1D1. For CR and PR, a scan must be performed at least 4 weeks after initial documentation of response to confirm the response. {X}: Only for Parts 2 and 4, radiologic follow-up will continue until disease progression, start of new therapy, withdrawal of consent, study closure, or death, whichever occurs first.
Study Treatment														
Pre-treatment		X				X				X				Refer to Section 6.1.3.
NL-201		X				X				X				
Parts 3 and 4 only: Pembrolizumab		X				X				X				To be administered prior to NL-201 (refer to Table 7 for details). Pembrolizumab may be administered up to 35 cycles.

AE = adverse event; CR = complete response; CT = computed tomography; CXDX = cycle X day X; D = day; ECG = electrocardiogram; ECHO = echocardiogram; ECOG = Eastern Cooperative Oncology Group; EOI = end of infusion; EOT = end of treatment; iRECIST = Response Evaluation Criteria in Solid Tumors for use in cancer immunotherapy trials; LTFU = long-term follow-up; M = month; MRI = magnetic resonance imaging; PD = progressive disease; PDn = pharmacodynamic(s); PD-L1 = programmed cell death ligand 1; PFTs = pulmonary function tests; PR = partial response; Q3W = every 3 weeks; RECIST = Response Evaluation Criteria in Solid Tumors; RFU = radiological follow-up; SAE = serious adverse event; SCR = screening; WOCBP = women of child bearing potential

a The screening period is up to 28 days prior Cycle 1 Day 1.

b All assessments should be performed pre-dose of NL-201 or pembrolizumab, if applicable unless otherwise indicated.

c Upon permanent discontinuation of study drug for any reason, an end of treatment visit should be scheduled as soon as possible but within 30 days following cessation of study treatment or before patient initiates new anti-cancer therapy, whichever is earlier.

d For Parts 1 and 3 (Dose Escalation), if study treatment is discontinued for reasons other than death, withdrawal of consent, lost to follow-up, or study closure, a CT scan should be performed at EOT visit (within + 2 weeks) if a post-baseline CT scan has not been performed within 5 weeks prior to the EOT visit.

e Long-term follow-up assessments occur every 6 months (± 1 month) until death, withdrawal of consent, lost to follow-up, or study closure, whichever occurs first, for a maximum of 2 years after end of treatment. This visit can occur by telephone.

Table 2 Schedule of Events – Schedule B (D1 & D8 Dosing)

	SCR[a]	Treatment Period[b] (21-day cycles)												EOT[c]	RFU	LTFU[e]	
		Cycle 1						Cycle 2				Cycle 3+					
		D1	D2	D4	D8	D9	D11	D1	D2	D4	D8	D1	D8				
Treatment Visit Window		0	0	0	±1	0	0	±3	0	0	±1	±3	±1				Notes
General and Safety Assessments																	
Informed consent	X																
Eligibility criteria	X																
Demography	X																
Medical history	X																
Physical examination	X	(X)			X			X			X	X		X			Full physical exam at screening and end of treatment; brief physical exam at all other time points (ie, C1D8, C2D8, and CXD1). (X): Not required if completed within 7 days of Day 1.
Height	X																
Weight	X	X			X			X			X	X	X	X			Dose adjustment required if weight changes ≥ 10% from weight used for C1D1 dose (recorded at screening or C1D1 visit or per institutional standard).
Vital signs	X	X	X		X			X			X	X	X	X			All D1 assessments include a pre-dose (within 1 h prior to dose) and a post-dose assessment 1 h after EOI (± 15 min). Measured in a semi-supine position after 5 min rest.
12-lead ECG	X	(X)						X						X			(X): Completed 6-24 hours after EOI
ECHO	X																If ECHO is not available or appropriate according to the institutional standard, then multi-gated acquisition (MUGA) scan is permitted
ECOG performance status	X	(X)						X				X		X			Must be confirmed per Section 8 before dosing on C1D1 (X): Assessment can be performed within 48 hours prior to dosing.
PFTs	X																
Hospitalization		X															24-hour hospitalization (may be extended per Investigator discretion). Patients should be advised to stay within a reasonable distance of the clinical site for the first week after the first dose.

	SCR[a]	Treatment Period[b] (21-day cycles)												EOT[c]	RFU	LTFU[e]	
		Cycle 1						Cycle 2				Cycle 3+					
		D1	D2	D4	D8	D9	D11	D1	D2	D4	D8	D1	D8				
Treatment Visit Window		0	0	0	±1	0	0	±3	0	0	±1	±3	±1				Notes
AE review	(X)	←											→				(X): Only collect AEs related to study-mandated procedures during the screening period. All AEs will be collected during the treatment period and followed for 30 days after the final dose of NL-201. All concomitant medications received within 28 days prior to the first dose of study treatment and up to 30 days after the last dose of study treatment will be recorded. In Parts 3 and 4, SAEs will be collected for 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anti-cancer therapy, whichever is earlier.
Concomitant medication review	←												→				
Survival status																X	
Parts 2 and 4: Subsequent anti-cancer therapy																X	
Local Laboratory Assessments – all samples collected pre-dose unless otherwise indicated																	
Pregnancy test	X	(X)						X				X		X			WOCBP only. Must be confirmed per Section 8 before dosing on C1D1. Pregnancy tests may be performed more frequently as required by local regulations. (X): Sample can be collected within 48 hours prior to dosing.
Viral serology	X																
Hematology	X	{X}	X	X	X	X	X	X	X	X	X	X	X	X			Hematology and chemistry laboratory tests must be confirmed per Section 8 before dosing on C1D1 {X}: Samples can be collected within 48 hours prior to dosing. [X]: Collected only if biopsy is performed.
Chemistry	X	{X}		X	X		X	X		X	X	X	X	X			
Coagulation	X	{X}			[X]			X			[X]	X		X			
Urinalysis	X	{X}			X			X				X		X			
Parts 3 and 4: Thyroid function tests	X	Every 6 weeks															

	SCR[a]	Treatment Period[b] (21-day cycles)												EOT[c]	RFU	LTFU[e]	
		Cycle 1						Cycle 2				Cycle 3+					
		D1	D2	D4	D8	D9	D11	D1	D2	D4	D8	D1	D8				
Treatment Visit Window		0	0	0	±1	0	0	±3	0	0	±1	±3	±1				Notes
Central Laboratory Assessments – all samples collected pre-dose unless otherwise indicated																	
Pharmacokinetics		(X)	X	X	(X)	X	X	(X)	X	X	X	X	X				All D1 and D8 collections include a pre-dose sample (within 1 h prior to dose of NL-201 or pembrolizumab, if applicable) and a post-dose sample 1 h after EOI (± 15 min) of NL-201. D2/D9 and D4/D11 collected 24 h and 72 h after EOI (± 4 h). (X): Additional post-NL-201 dose collections at following time points: • At EOI (+ 10 min) • 2 h after EOI (± 20 min) • 4 h and 8 h after EOI (± 30 min)
Antidrug antibody		X			X			X			X	X		X			
Cytokines		(X)	X	X	(X)	X	X	(X)	X	X	X	X	X				All D1 and D8 collections include a pre-dose sample (within 1 h prior to dose of NL-201 or pembrolizumab, if applicable) and a post-dose sample 1 h after EOI (± 15 min) of NL-201. D2/D9 and D4/D11 collected 24 and 72 h after EOI (± 4 h). (X): Additional post-dose collection 4h after EOI (± 30 min)
PDn blood samples		X	X	X	X	X	X	X	X	X	X	X	X				
Optional Part 1 & 2: Tumor biopsy (for PD analyses)	X										X						Patients who consent to tumor biopsy with readily accessible tumor tissue will have a biopsy performed at screening up to 28 days prior to the first dose (C1D1). Patients will have a 2nd paired biopsy 5 to 9 days after receiving dose at Cycle 2 Day 1 (C2D1) before the dosing at Cycle 2 Day 8 (C2D8) visit. Note: Biopsy may be collected on the same day as dosing if completed before any dosing begins.
Part 4: Tumor tissue (for PD-L1)	X																Archival biopsy should be within 5 years of screen date for PD-L1 analysis. If archival tissue not available, perform fresh tumor biopsy if readily accessible.

	SCR[a]	Treatment Period[b] (21-day cycles)												EOT[c]	RFU	LTFU[e]	
		Cycle 1						Cycle 2				Cycle 3+					
		D1	D2	D4	D8	D9	D11	D1	D2	D4	D8	D1	D8				
Treatment Visit Window		0	0	0	±1	0	0	±3	0	0	±1	±3	±1				Notes
Part 4: Tumor biopsy (for PD analyses)	X										X			(X)			Part 4 patients with readily accessible tumor tissue will have a biopsy performed at screening up to 28 days prior to the first dose (C1D1). Patients will have a 2nd paired biopsy 7 (± 2) days after the Cycle 2 dose. (X): EOT collection is optional.
Efficacy Assessments																	
CT scan	(X)	Weeks 1-12: Every 6 weeks (± 3d) Weeks 13+: Every 12 weeks (± 7d)												X[d]	{X}		All scans are calculated from C1D1. RECIST 1.1 and iRECIST assessments continue until disease progression (even if study treatment is discontinued). MRI required only to confirm brain metastasis, if applicable. (X): Completed within 28 days of C1D1. For CR and PR, a scan must be performed at least 4 weeks after initial documentation of response to confirm the response. {X}: Only for Parts 2 and 4, radiologic follow-up will continue until disease progression, start of new therapy, withdrawal of consent, study closure, or death, whichever occurs first

	SCR[a]	Treatment Period[b] (21-day cycles)												EOT[c]	RFU	LTFU[e]	
		Cycle 1						Cycle 2				Cycle 3+					
		D1	D2	D4	D8	D9	D11	D1	D2	D4	D8	D1	D8				
Treatment Visit Window		0	0	0	±1	0	0	±3	0	0	±1	±3	±1				Notes
Study Treatment																	
Pre-treatment		X			X			X			X	X	X				Refer to Section 6.1.3.
NL-201 IV		X			X			X			X	X	X				
Parts 3 and 4 only: Pembrolizumab		X						X				X					To be administered prior to NL-201 (refer to Table 7 for details). Pembrolizumab may be administered up to 35 cycles.

AE = adverse event; CR = complete response; CT = computed tomography; CXDX = cycle X day X; D = day; ECG = electrocardiogram; ECHO = echocardiogram; ECOG = Eastern Cooperative Oncology Group; EOI = end of infusion; EOT = end of treatment; iRECIST = Response Evaluation Criteria in Solid Tumors for use in cancer immunotherapy trials; IV = intravenous; LTFU = long-term follow-up; M = month; MRI = magnetic resonance imaging; PD = progressive disease; PD-L1 = programmed cell death ligand 1; PDn = pharmacodynamic(s); PFTs = pulmonary function tests; PR = partial response; RECIST = Response Evaluation Criteria in Solid Tumors; RFU = radiological follow-up; SAE = serious adverse event; SCR = screening; WOCBP = women of child bearing potential

a The screening period is up to 28 days prior to Cycle 1 Day 1.

b All assessments should be performed pre-dose of NL-201 or pembrolizumab, if applicable unless otherwise indicated.

c Upon permanent discontinuation of study drug for any reason, an end of treatment visit should be scheduled as soon as possible but within 30 days following cessation of study treatment or before patient initiates new anti-cancer therapy, whichever is earlier.

d For Parts 1 and 3 (Dose Escalation), if study treatment is discontinued for reasons other than death, withdrawal of consent, lost to follow-up, or study closure, a CT scan should be performed at EOT visit (within + 2 weeks) if a post-baseline CT scan has not been performed within 5 weeks prior to the EOT visit.

e Long-term follow-up assessments occur every 6 months (± 1 month) until death, withdrawal of consent, lost to follow-up, or study closure, whichever occurs first, for a maximum of 2 years after end of treatment. This visit can occur by telephone.

Table 3 Schedule of Events – Schedule A (Q3W Dosing) if Step-Dosing is Implemented

	SCR[a]	Treatment Period[b] (21-day cycles)									EOT[c]	RFU	LTFU[e]	
		Cycle 1				Cycle 2				Cycle 3+				
		D1	D2	D4	D8	D1	D2	D4	D8	D1				
Treatment Visit Window		0	0	0	±1	±3	0	0	±1	±3				Notes
General and Safety Assessments														
Informed consent	X													
Eligibility criteria	X													
Demography	X													
Medical history	X													
Physical examination	X	(X)			X	X				X	X			Full physical exam at screening and end of treatment; brief physical exam at all other time points (ie, C1D8 and CXD1). (X): Not required if completed within 7 days of Day 1.
Height	X													
Weight	X	X			X	X				X	X			Dose adjustment required if weight changes ≥ 10% from weight used for C1D1 dose (recorded at screening or C1D1 visit or per institutional standard).
Vital signs	X	X	X		X	X				X	X			All D1 assessments include a pre-dose (within 1 h prior to dose) and a post-dose assessment 1h after EOI (± 15 min). Measured in a semi-supine position after 5 min rest.
12-lead ECG	X	(X)				X					X			(X): Completed 6-24 hours after EOI
ECHO	X													If ECHO is not available or appropriate according to the institutional standard, then multi-gated acquisition (MUGA) scan is permitted
ECOG performance status	X	(X)				X				X	X			Must be confirmed per Section 8 before dosing on C1D1 (X): Assessment can be performed within 48 hours prior to dosing.
PFTs	X													
Hospitalization		X												24-hour hospitalization (may be extended per Investigator discretion). Patients should be advised to stay within a reasonable distance of the clinical site for the first week after the first dose.

	SCR[a]	Treatment Period[b] (21-day cycles)									EOT[c]	RFU	LTFU[c]	Notes
		Cycle 1				Cycle 2				Cycle 3+				
		D1	D2	D4	D8	D1	D2	D4	D8	D1				
Treatment Visit Window		0	0	0	±1	±3	0	0	±1	±3				
AE review	(X)	←═══								→				(X): Only collect AEs related to study-mandated procedures during the screening period. All AEs will be collected during the treatment period and followed for 30 days after the final dose of NL-201. All concomitant medications received within 28 days prior to the first dose of study treatment and up to 30 days after the last dose of study treatment will be recorded. In Parts 3 and 4, SAEs will be collected for 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anti-cancer therapy, whichever is earlier.
Concomitant medication review		←═══								→				
Survival status													X	
Parts 2 and 4: Subsequent anti-cancer therapy													X	
Local Laboratory Assessments – all samples collected pre-dose unless otherwise indicated														
Pregnancy test	X	(X)				X				X	X			WOCBP only. Must be confirmed per Section 8 before dosing on C1D1. Pregnancy tests may be performed more frequently as required by local regulations (X): Sample can be collected within 48 hours prior to dosing
Viral serology	X													
Hematology	X	{X}	X	X	X	X	X	X		X	X			Hematology and chemistry laboratory tests must be confirmed per Section 8 before dosing on C1D1 {X}: Samples can be collected within 48 hours prior to dosing. [X]: Collected only if biopsy is performed.
Chemistry	X	{X}		X	X	X		X		X	X			
Coagulation	X	{X}			[X]	X			[X]	X	X			
Urinalysis	X	{X}			X	X				X	X			
Parts 3 and 4: Thyroid function tests	X	Every 6 weeks												

	SCR[a]	Treatment Period[b] (21-day cycles)									EOT[c]	RFU	LTFU[c]	
		Cycle 1				Cycle 2				Cycle 3+				
		D1	D2	D4	D8	D1	D2	D4	D8	D1				
Treatment Visit Window		0	0	0	±1	±3	0	0	±1	±3				Notes
Central Laboratory Assessments – all samples collected pre-dose unless otherwise indicated														
Pharmacokinetics		(X)	X	X	(X)	(X)	X	X		X				All D1 and D8 collections include a pre-dose sample (within 1 h prior to dose of NL-201 or pembrolizumab, if applicable) and a post-dose sample 1 h after EOI (± 15 min) of NL-201. D2 and D4 collected 24 h and 72 h after EOI (± 4 h). (X): Additional post-dose collections at following time points: • At EOI (+ 10 min) • 2 h after EOI (± 20 min) • 4 h and 8 h after EOI (± 30 min)
Antidrug antibody		X			X	X				X	X			
Cytokines		(X)	X	X	X	(X)	X	X		X				All D1 and D8 collections include a pre-dose sample (within 1 h prior to dose of NL-201 or pembrolizumab, if applicable) and a post-dose sample 1 h after EOI (± 15 min) of NL-201. D2 and D4 collected 24 h and 72 h after EOI (± 4 h). (X): Additional post-dose collection 4 h after EOI (± 30 min)
PDn blood samples		X	X	X	X	X	X	X		X				
Optional Part 1 & 2: Tumor biopsy (for PD analyses)	X								X					Patients who consent to tumor biopsy with readily accessible tumor tissue will have a biopsy performed at screening up to 28 days prior to the first dose (C1D1). Patients will have a 2nd paired biopsy 5 to 9 days after receiving dose at Cycle 2 Day 1 (C2D1).
Part 4: Tumor tissue (for PD-L1)	X													Archival biopsy should be within 5 years of screen date for PD-L1 analysis. If archival tissue not available, perform fresh tumor biopsy if readily accessible.
Part 4: Tumor biopsy (for PD analyses)	X								X		(X)			Part 4 patients with readily accessible tumor tissue will have a biopsy performed at screening up to 28 days prior to the first dose (C1D1). Patients will have a 2nd paired biopsy 7 (± 2) days after the Cycle 2 dose. (X): EOT collection is optional.

	SCR[a]	Treatment Period[b] (21-day cycles)									EOT[c]	RFU	LTFU[c]	
		Cycle 1				Cycle 2				Cycle 3+				
		D1	D2	D4	D8	D1	D2	D4	D8	D1				
Treatment Visit Window		0	0	0	±1	±3	0	0	±1	±3				Notes
Efficacy Assessments														
CT scan	(X)	Weeks 1-12: Every 6 weeks (± 3d) Weeks 13+: Every 12 weeks (± 7d)									X[d]	{X}		All scans are calculated from C1D1. RECIST 1.1 and iRECIST assessments continue until disease progression (even if study treatment is discontinued). MRI required only to confirm brain metastasis, if applicable. (X): Completed within 28 days of C1D1. For CR and PR, a scan must be performed at least 4 weeks after initial documentation of response to confirm the response. {X}: Only for Parts 2 and 4, radiologic follow-up will continue until disease progression, start of new therapy, withdrawal of consent, study closure, or death, whichever occurs first
Study Treatment														
Pre-treatment		X			X	X				X				Refer to Section 6.1.3.
NL-201: 1 step		X*			X*	X				X				X*: Refer to Table 10 if step-dosing is implemented.
NL-201: 2 steps		X*			X*	X*				X				
NL-201: 3 steps		X*			X*	X*				X*				
Parts 3 and 4: pembrolizumab		X				X				X				To be administered prior to NL-201 (refer to Table 7 for details). Pembrolizumab may be administered up to 35 cycles.

	SCR[a]	Treatment Period[b] (21-day cycles)									EOT[c]	RFU	LTFU[e]	
		Cycle 1				Cycle 2				Cycle 3+				
		D1	D2	D4	D8	D1	D2	D4	D8	D1				
Treatment Visit Window		0	0	0	±1	±3	0	0	±1	±3				Notes

AE = adverse event; CR = complete response; CT = computed tomography; CXDX = cycle X day X; D = day; ECG = electrocardiogram; ECHO = echocardiogram; ECOG = Eastern Cooperative Oncology Group; EOI = end of infusion; EOT = end of treatment; iRECIST = Response Evaluation Criteria in Solid Tumors for use in cancer immunotherapy trials; LTFU = long-term follow-up; M = month; MRI = magnetic resonance imaging; PD = progressive disease; PD-L1 = programmed cell death ligand 1; PDn = pharmacodynamic(s); PFTs = pulmonary function tests; PR = partial response; Q3W = every 3 weeks; RECIST = Response Evaluation Criteria in Solid Tumors; RFU = radiological follow-up; SAE = serious adverse event; SCR = screening; WOCBP = women of child bearing potential

a The screening period is up to 28 days prior to Cycle 1 Day 1.

b All assessments should be performed pre-dose of NL-201 or pembrolizumab, if applicable unless otherwise indicated.

c Upon permanent discontinuation of study drug for any reason, an end of treatment visit should be scheduled as soon as possible but within 30 days following cessation of study treatment or before patient initiates new anti-cancer therapy, whichever is earlier.

d For Parts 1 and 3 (Dose Escalation), if study treatment is discontinued for reasons other than death, withdrawal of consent, lost to follow-up, or study closure, a CT scan should be performed at EOT visit (within + 2 weeks) if a post-baseline CT scan has not been performed within 5 weeks prior to the EOT visit.

e Long-term follow-up assessments occur every 6 months (± 1 month) until death, withdrawal of consent, lost to follow-up, or study closure, whichever occurs first, for a maximum of 2 years after end of treatment. This visit can occur by telephone.

Table 4 Schedule of Events – Schedule B (D1 & D8 Dosing) if Step-Dosing is Implemented

	SCR[a]	Treatment Period[b] (21-day cycles)												EOT[c]	RFU	LTFU[e]	
		Cycle 1						Cycle 2				Cycle 3+					
		D1	D2	D4	D8	D9	D11	D1	D2	D4	D8	D1	D8				
Treatment Visit Window		0	0	0	±1	0	0	+3	0	0	±1	±3	±1				Notes
General and Safety Assessments																	
Informed consent	X																
Eligibility criteria	X																
Demography	X																
Medical history	X																
Physical examination	X	(X)			X			X			X	X		X			Full physical exam at screening and end of treatment; brief physical exam at all other time points (ie, C1D8, C2D8, and CXD1). (X): Not required if completed within 7 days of Day 1.
Height	X																
Weight	X	X			X			X			X	X	X	X			Dose adjustment required if weight changes ≥ 10% from weight used for C1D1 dose (recorded at screening or C1D1 visit or per institutional standard).
Vital signs	X	X	X		X			X			X	X	X	X			All D1 assessments include a pre-dose (within 1 h prior to dose) and a post-dose assessment 1 h after EOI (± 15 min). Measured in a semi-supine position after 5 min rest.
12-lead ECG	X	(X)						X						X			(X): Completed 6-24 hours after EOI
ECHO	X																If ECHO is not available or appropriate according to the institutional standard, then multi-gated acquisition (MUGA) scan is permitted
ECOG performance status	X	(X)						X				X		X			Must be confirmed per Section 8 before dosing C1D1 (X): Assessment can be performed within 48 hours prior to dosing
PFTs	X																
Hospitalization		X															24-hour hospitalization (may be extended per Investigator discretion). Patients should be advised to stay within a reasonable distance of the clinical site for the first week after the first dose.

	SCR[a]	Treatment Period[b] (21-day cycles)												EOT[c]	RFU	LTFU[e]	Notes
		Cycle 1						Cycle 2				Cycle 3+					
		D1	D2	D4	D8	D9	D11	D1	D2	D4	D8	D1	D8				
Treatment Visit Window		0	0	0	±1	0	0	+3	0	0	±1	±3	±1				
AE review	(X)	←											→				(X): Only collect AEs related to study-mandated procedures during the screening period. All AEs will be collected during the treatment period and followed for 30 days after the final dose of NL-201. All concomitant medications received within 28 days prior to the first dose of study treatment and up to 30 days after the last dose of study treatment will be recorded. In Parts 3 and 4, SAEs will be collected for 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anti-cancer therapy, whichever is earlier.
Concomitant medication review		←											→				
Survival status																X	
Parts 2 and 4: Subsequent anti-cancer therapy																X	
Local Laboratory Assessments – all samples collected pre-dose unless otherwise indicated																	
Pregnancy test	X	(X)						X				X		X			WOCBP only. Must be confirmed per Section 8 before dosing on C1D1. Pregnancy tests may be performed more frequently as required by local regulations (X): Sample can be collected within 48 hours prior to dosing
Viral serology	X																
Hematology	X	{X}	X	X	X	X	X	X	X	X	X	X	X	X			Hematology and chemistry laboratory tests must be confirmed per Section 8 before dosing on C1D1 {X}: Samples can be collected within 48 hours prior to dosing. [X]: Collected only if biopsy is performed.
Chemistry	X	{X}		X	X		X	X		X	X	X	X	X			
Coagulation	X	{X}			[X]			X			[X]	X		X			
Urinalysis	X	{X}			X			X				X		X			
Parts 3 and 4: Thyroid function tests	X	Every 6 weeks															

	SCR[a]	Treatment Period[b] (21-day cycles)												EOT[c]	RFU	LTFU[e]	
		Cycle 1						Cycle 2				Cycle 3+					
		D1	D2	D4	D8	D9	D11	D1	D2	D4	D8	D1	D8				
Treatment Visit Window		0	0	0	±1	0	0	+3	0	0	±1	±3	±1				Notes
Central Laboratory Assessments – all samples collected pre-dose unless otherwise indicated																	
Pharmacokinetics		(X)	X	X	(X)	X	X	(X)	X	X	X	X	X				All D1 and D8 collections include a pre-dose sample (within 1 h prior to dose of NL-201 or pembrolizumab, if applicable) and a post-dose sample 1 h after EOI (± 15 min) of NL-201. D2/D9 and D4/D11 collected 24 h and 72 h after EOI (± 4 h). (X): Additional post-NL-201 dose collections at following time points: • At EOI (+ 10 min) • 2 h after EOI (± 20 min) • 4 h and 8 h after EOI (± 30 min)
Antidrug antibody		X			X			X			X	X		X			
Cytokines		(X)	X	X	(X)	X	X	(X)	X	X	X	X	X				All D1 and D8 collections include a pre-dose sample (within 1 h prior to dose of NL-201 or pembrolizumab, if applicable) and a post-dose sample 1 h after EOI (± 15 min) of NL-201. D2/D9 and D4/D11 collected 24 and 72 h after EOI (± 4 h). (X): Additional post-dose collection 4 h after EOI (± 30 min)
PDn blood samples		X	X	X	X	X	X	X	X	X	X	X	X				
Optional Part 1 & 2: Tumor biopsy (for PD analyses)	X										X						Patients who consent to tumor biopsy with readily accessible tumor tissue will have a biopsy performed at screening up to 28 days prior to the first dose (C1D1). Patients will have a 2nd paired 5 to 9 days after receiving dose at Cycle 2 Day 1 (C2D1), before the dosing at Cycle 2 Day 8 (C2D8) visit. Note: Biopsy may be collected on the same day as dosing if completed before any dosing begins.
Part 4: Tumor tissue (for PD-L1)	X																Archival biopsy should be within 5 years of screen date for PD-L1 analysis. If archival tissue not available, perform fresh tumor biopsy if readily accessible.

	SCR[a]	Treatment Period[b] (21-day cycles)												EOT[c]	RFU	LTFU[e]	Notes
		Cycle 1						Cycle 2				Cycle 3+					
		D1	D2	D4	D8	D9	D11	D1	D2	D4	D8	D1	D8				
Treatment Visit Window		0	0	0	±1	0	0	+3	0	0	±1	±3	±1				
Part 4: Tumor biopsy (for PD analyses)	X										X			(X)			Part 4 patients with readily accessible tumor tissue will have a biopsy performed at screening up to 28 days prior to the first dose (C1D1). Patients will have a 2nd paired biopsy 7 (± 2) days after the Cycle 2 dose. (X): EOT collection is optional.
Efficacy Assessments																	
CT scan	(X)	Weeks 1-12: Every 6 weeks (± 3d) Weeks 13+: Every 12 weeks (± 7d)												X[d]	{X}		All scans are calculated from C1D1. RECIST 1.1 and iRECIST assessments continue until disease progression (even if study treatment is discontinued). MRI required only to confirm brain metastasis, if applicable. (X): Completed within 28 days of C1D1. For CR and PR, a scan must be performed at least 4 weeks after initial documentation of response to confirm the response. {X}: Only for Parts 2 and 4, radiologic follow-up will continue until disease progression, start of new therapy, withdrawal of consent, study closure, or death, whichever occurs first

	SCR[a]	Treatment Period[b] (21-day cycles)												EOT[c]	RFU	LTFU[e]	
		Cycle 1						Cycle 2				Cycle 3+					
		D1	D2	D4	D8	D9	D11	D1	D2	D4	D8	D1	D8				
Treatment Visit Window		0	0	0	±1	0	0	+3	0	0	±1	±3	±1				Notes
Study Treatment																	
Pre-treatment		X			X			X			X	X	X				Refer to Section 6.1.3.
NL-201: 1 step		X*			X*			X			X	X	X				X*: Refer to Table 10 if step-dosing is implemented.
NL-201: 2 steps		X*			X*			X*			X*	X	X				
NL-201: 2 steps		X*			X*			X*			X*	X*	X*				
Parts 3 and 4: Pembrolizumab		X						X				X					To be administered prior to NL-201 (refer to Table 7 for details). Pembrolizumab may be administered up to 35 cycles.

AE = adverse event; CR = complete response; CT = computed tomography; CXDX = cycle X day X; D = day; ECG = electrocardiogram; ECHO = echocardiogram; ECOG = Eastern Cooperative Oncology Group; EOI = end of infusion; EOT = end of treatment; iRECIST = Response Evaluation Criteria in Solid Tumors for use in cancer immunotherapy trials; LTFU = long-term follow-up; M = month; MRI = magnetic resonance imaging; PD = progressive disease; PDn = pharmacodynamic(s); PFTs = pulmonary function tests; PR = partial response; RECIST = Response Evaluation Criteria in Solid Tumors; RFU = radiological follow-up; SAE = serious adverse event; SCR = screening; WOCBP = women of child bearing potential

a The screening period is up to 28 days prior to Cycle 1 Day 1.

b All assessments should be performed pre-dose of NL-201 or pembrolizumab, if applicable unless otherwise indicated.

c Upon permanent discontinuation of study drug for any reason, an end of treatment visit should be scheduled as soon as possible but within 30 days following cessation of study treatment or before patient initiates new anti-cancer therapy, whichever is earlier.

d For Parts 1 and 3 (Dose Escalation), if study treatment is discontinued for reasons other than death, withdrawal of consent, lost to follow-up, or study closure, a CT scan should be performed at EOT visit (within + 2 weeks) if a post-baseline CT scan has not been performed within 5 weeks prior to the EOT visit.

e Long-term follow-up assessments occur every 6 months (± 1 month) until death, withdrawal of consent, lost to follow-up, or study closure, whichever occurs first, for a maximum of 2 years after end of treatment. This visit can occur by telephone.

2 INTRODUCTION

NL-201 is a novel, potent, and selective long-acting agonist of the beta and gamma receptors of the interleukin (IL)-2 receptor that is being developed as cancer immunotherapy.

2.1 Study Rationale

A majority of patients with relapsed or refractory solid tumors, who have failed available approved lines of therapies, represent a significant unmet medical need. IL-2 and IL-15 are immune agonists that may elicit antitumor responses in T-cells and natural killer (NK) cells. NL-201 is a novel therapeutic that triggers signaling in both T-cells and NK cells through the IL-2 receptor and may be less toxic than native proteins due to eliminating bias towards off target cells. Among the range of solid cancers that may respond to an immune agonist like NL-201, renal cell carcinoma (RCC) and melanoma cancer are of special interest because recombinant human IL-2 (aldesleukin) has been shown to benefit some patients with these malignancies. Standard of care for malignant melanoma and RCC includes immunotherapy with checkpoint inhibitors (CPIs). However, most patients progress or do not tolerate these therapies, and new modalities for the treatment of relapsed or refractory malignant melanoma and RCC are needed.[1] Thus, NL-201-101 will evaluate the benefit/risk profile of NL-201 in patients with relapsed or refractory solid tumors.

For the majority of cancer patients, new therapies are urgently needed that can turn cold tumors, tumors that have low numbers T-cell infiltrates, hot, leading to infiltration and activation of immune cells into the tumor microenvironment (TME). In pre-clinical models, NL-201 has been shown to increase T-cell receptor diversity in the TME, and to turn cold tumors hot. The combination of NL-201 with CPIs has shown marked additive and/or synergistic activity in multiple preclinical models. Thus, it is of interest to test this combination in patients as a potential novel regimen.

2.2 Background

2.2.1 Disease Background

Relapsed or refractory advanced cancer remains a major health problem worldwide, ranking second to cardiovascular disease as an overall cause of mortality according to the World Health Organization. Although there has been significant progress over the last few decades, patients with relapsed or refractory advanced solid tumors still have a generally poor prognosis. Among the range of solid cancers that may respond to an immune agonist like NL-201, RCC and melanoma cancer are of special interest, though patients with other tumor types who have exhausted available therapies may also participate in the proposed dose escalation study.

2.2.1.1 Renal Cell Carcinoma

RCC are tumors of the epithelium of the kidney, and they represent the most common cancer of the kidneys in the United States. Approximately 75% of RCC are classified as clear cell

carcinoma, with papillary (15%) and chromophobe (5%) types comprising the bulk of the remaining cases. The prognosis of advanced RCC is poor.

The pathogenesis of RCC includes impairment of host immune function, with an up-regulation of regulatory T-cells and myeloid-derived suppressor cells, as well as down-regulation of T-cell mediated immunity.[2] Immunotherapies that activate T-cell mediated antitumor activity, including IL-2, can counteract this pathogenic immune dysregulation, and have been shown to be effective in the treatment of malignant RCC. To this end, IL-2 has been used for patients with metastatic clear cell RCC. Clinical studies of high-dose IL-2 yielded durable complete responses (CRs) in 7% to 28% of patients, with a median duration of approximately 19 months,[2] and its use is warranted in patients with low-volume disease, good performance status, and predominantly clear cell carcinoma. Unfortunately, high-dose IL-2 is known to cause significant dose-related morbidity. Common serious adverse effects of high-dose IL-2 therapy include hypotension, pulmonary edema and capillary leak syndrome, wherein fluid accumulates in the extravascular space resulting in hypovolemia, oliguria, ischemia, and confusion. Other toxicities involve the heart, lungs, kidneys, and central nervous system (CNS). This toxicity profile has limited the use of high-dose IL-2 to specialized medical centers with experienced medical providers, where mortality can generally be minimized. Despite these risks, IL-2 can significantly benefit a modest number of patients.

2.2.1.2 Malignant Melanoma

Melanoma is 1 of the most common cancers in the United States, and is the most morbid malignancy of the skin, accounting for up to 75% of skin cancer-related deaths. While early melanoma is a localized disease, and is most often curable by surgical resection before metastatic spread, disseminated melanoma is a difficult disease to treat effectively.

Melanoma is a highly immunogenic tumor due to somatic mutations in the genomes of melanoma cells caused by ultraviolet radiation. These somatic mutations cause neoantigens in the tumor that can be recognized by the immune system, enabling a T-cell mediated antitumor immune response. Thus, immunotherapy can be an effective treatment for melanoma. Recent advances with CPIs have increased the 3-year survival rate of advanced melanoma to as high as 58%,[3] but CR rates are generally low, and recurrent and refractory disease remains an unmet medical need. High-dose IL-2 can also potentiate T-cell mediated antitumor responses in melanoma, with an objective response rate (ORR) of 16%, including a CR rate of 6%.[4] As described above, the severe toxicity associated with IL-2 therapy has limited its use to patients with good organ function who are being closely monitored by experienced clinicians in specialized medical centers.

2.2.2 Neoleukin Investigational Product Background: NL-201

NL-201 is a de novo computationally designed protein that is conjugated to a single polyethylene glycol (PEG) molecule and is a mimetic of the natural cytokines IL-2 and IL-15. The protein was originally designed using canonical amino acids (excluding cysteine).[5] Briefly, the crystallographic holo-structure of human IL-2 in complex with its receptor was used to derive the position and orientation of critical amino acids that mediate binding to IL-2 receptor beta and

gamma (but not IL-2 receptor alpha). These structural data were used to design a computationally de novo protein that was predicted to recapitulate these critical binding amino acids using a novel sequence and structural topology. As this protein was designed de novo, it has no significant homology to any protein found in nature.

After initial computational design, the protein was optimized by selecting high affinity and stability variants from a single-site saturation mutagenesis library. Multiple enriching mutations from this selection were combined to generate the final protein, named Neoleukin-2/15 (Neo-2/15). Neo-2/15 was found to have nanomolar binding affinity for both the human and murine IL-2 receptor beta and gamma, while having no binding affinity of any kind for human or murine IL-2 receptor alpha. The predicted structure of Neo-2/15 in complex with murine IL-2 receptor was confirmed by X-ray crystallography.

NL-201 was created by mutating a single amino-acid in the third helical element of Neo-2/15 (that does not interact with IL-2 receptor) to cysteine, and conjugating a single 40 kDa linear PEG molecule to that cysteine using maleimide chemistry. It behaves similarly to Neo-2/15 in that it binds the same receptors with similar affinity and triggers signal transducer and activator of transcription 5 (STAT5) signaling on human cells with similar potency but is expected to have a longer serum half-life ($t_{1/2}$) due to decreased renal filtration.

Neo-2/15 triggers phosphorylation of STAT5 with an 50% effective concentration of approximately 10^{-11} M. Ex vivo, it drives proliferation of primary murine derived T-cells as well as of murine CTLL-2 cell lines. *In vivo*, NL-201 causes expansion of multiple lymphoid cell lineages, including T-cells, and in murine models is an efficacious treatment for CT-26 colon cancer.

Safety studies of NL-201 involved no unscheduled deaths and no significant macroscopic findings and identified a no observed adverse effect level (NOAEL) of 11.44 µg/kg in non-human primates. Microscopic findings at terminal necropsy showed moderate dose-dependent mixed leukocyte infiltration in multiple tissues, including the choroid plexus of the brain, glandular organs, urogenital and reproductive tract, heart, kidneys, injection site, gastrointestinal tract and skin. Leukocytic infiltration caused by higher doses of NL-201 led to minimal to mild myofiber degradation and necrosis and minimal to mild renal tubular degeneration. All changes at 11.44 µg/kg were less severe after 4 weeks of recovery, consistent with partial or full reversibility.

A detailed description of the chemistry, pharmacology, and safety of NL-201 is provided in the Investigator's Brochure.

2.2.3 Non-Neoleukin Investigational Product: Pembrolizumab

Pembrolizumab is a potent humanized immunoglobulin G4 (IgG4) monoclonal antibody (mAb) with high specificity of binding to the programmed cell death 1 (PD-1) receptor, thus inhibiting its interaction with programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2). Based on preclinical *in vitro* data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1. Pembrolizumab has an acceptable preclinical safety profile and is in clinical development as an IV immunotherapy for advanced malignancies. Keytruda®

(pembrolizumab) is indicated for the treatment of patients across a number of indications. For more details on specific indications refer to the Investigator's Brochure.

Refer to the Investigator's Brochure/approved labeling for detailed background information on pembrolizumab.

2.2.3.1 Pharmaceutical and Therapeutic Background

The importance of intact immune surveillance function in controlling outgrowth of neoplastic transformations has been known for decades.[6] Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of cluster of differentiation (CD)8+ T-cells and the ratio of CD8+ effector T-cells/forkhead box P3 (FoxP3)+ regulatory T-cells (T-regs) correlates with improved prognosis and long-term survival in solid malignancies, such as ovarian, colorectal, and pancreatic cancer; hepatocellular carcinoma; malignant melanoma; and RCC. Tumor-infiltrating lymphocytes can be expanded ex vivo and reinfused, inducing durable objective tumor responses in cancers such as melanoma.[7,8]

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an immunoglobulin (Ig) superfamily member related to CD28 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) that has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2).[9,10]

The structure of murine PD-1 has been resolved.[11] PD-1 and its family members are type I transmembrane glycoproteins containing an Ig-variable–type (IgV-type) domain responsible for ligand binding and a cytoplasmic tail responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif, and an immunoreceptor tyrosine-based switch motif. Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases, SHP-1 and SHP-2, to the immunoreceptor tyrosine-based switch motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3 zeta (CD3ζ), protein kinase C-theta (PKCθ), and zeta-chain-associated protein kinase (ZAP70), which are involved in the CD3 T-cell signaling cascade.[10,12–14] The mechanism by which PD-1 down-modulates T-cell responses is similar to, but distinct from, that of CTLA-4, because both molecules regulate an overlapping set of signaling proteins.[15,16] As a consequence, the PD-1/PD-L1 pathway is an attractive target for therapeutic intervention in patients with relapsed or refractory cancer.

2.2.3.2 Pre-Clinical and Clinical Trials

Therapeutic studies in mouse models have shown that administration of antibodies blocking PD-1/PD-L1 interaction enhances infiltration of tumor-specific CD8+ T-cells and ultimately leads to tumor rejection, either as a monotherapy or in combination with other treatment modalities.[17–23] Anti-mouse PD-1 or anti-mouse PD-L1 antibodies have demonstrated antitumor responses in models of squamous cell carcinoma, pancreatic carcinoma, melanoma, acute

myeloid leukemia and colorectal carcinoma.[11,20,22–24] In such studies, tumor infiltration by CD8+ T-cells and increased interferon gamma (IFN-γ), granzyme B and perforin expression were observed, indicating that the mechanism underlying the antitumor activity of PD-1 checkpoint inhibition involved local infiltration and activation of effector T-cell function *in vivo*.[22] Experiments have confirmed the *in vivo* efficacy of anti-mouse PD-1 antibody as a monotherapy, as well as in combination with chemotherapy, in syngeneic mouse tumor models (see the Investigator's Brochure).

2.3 Benefit/Risk Assessment

Potential risks for the planned NL201-101 first-in-human clinical study comprise cytokine release syndrome, flu like symptoms, cardiac toxicity, and auto-immunity. Considering the high unmet medical need in the targeted patient population and that patients do not forego any established and effective standard therapy, the risk/benefit ratio of applying an engineered IL-2 mimetic with potential for durable responses that is safer than IL-2 is considered justified by the Sponsor. To further lower the safety risks for patients in the NL201-101 study, risk mitigation measures have been implemented at multiple levels in the design of the clinical study. These include dose escalation/de-escalation, prophylactic measures, steps that allow for early diagnosis and/or aggressive clinical management of safety events, and a Data Monitoring Committee (DMC).

More detailed information about the known and expected benefits and risks and reasonably expected adverse events (AEs) of NL-201 may be found in the Investigator's Brochure.

2.3.1 Risk Assessment

Table 5 Risk Assessment

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Study drug(s)		
In addition to the specific risks described below, unknown risks may be associated with a first-in-human application (eg, allergic reactions, infusion-related reactions [chills/rigors, fever, headache, pruritus, rash, arthralgia, myalgia, bronchospasm, hypotension, other hypersensitivity/allergic-like reactions])		• Study design: A dose escalation/de-escalation schedule and stepwise enrollment of the first patient in each cohort has been introduced into the clinical protocol. The DMC will review safety data on a regular basis and ad hoc if required. • Patient selection: Prostate cancer, a common cancer with low potential to respond to immunotherapy, is excluded to decrease risk to patients with low likelihood of benefit. • Early diagnosis of potential toxicities: On D1 of the study, patients will receive IV infusion of NL-201 in a hospital setting and will be monitored for 24 hours. • Prevention: Patients may receive prophylaxis against allergic reactions per Section 6.1.3 and will be closely monitored in the hours following the infusion. • Clinical management for infusion-related reactions: Refer to Table 11 for guidance.
Exacerbation of HIV	IL-2 and IL-15 can induce HIV viral replication and increase CD4 T-cell susceptibility to HIV.	• Patient selection: Patients are screened for HIV during screening, and HIV positive patient are excluded from this first-in-human study. Investigation of the safety of NL-201 in HIV positive patients will be considered in future studies when the safety profile of the drug has been determined.
Cytokine release syndrome (fever, chills/rigors, vomiting, tachycardia, headache, rash, arthralgia, myalgia, chest discomfort, angioedema, hypotension, dyspnea)	Systemic high dose IL-2 therapy has been known to cause cytokine release syndrome, though the cytokine profile of NL-201 does not appear consistent with classic cytokine release.	• Patient monitoring: Patients are dosed in a hospital setting with availability of an ICU • Prevention: Patients may receive pre-medication per Section 6.1.3. • Clinical management: Depending on the severity of symptoms, patients with evidence of cytokine release syndrome may have pre-medication adjusted (per Section 6.1.3) may be dose-reduced or receive no further doses of NL-201 (refer to Table 11 for guidance) and pending discussion with medical monitor. Cytokine release syndrome should be managed per Investigator discretion and according to institutional guidelines, eg, pulmonary edema can be managed with standard clinical support, including supplemental oxygen or positive pressure ventilation; lower extremity edema can be managed with standard compression stockings, if appropriate; renal toxicity can be managed with fluid resuscitation.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Flu-like symptoms (fever, chills, myalgia, malaise, arthralgia, fatigue, headache, nausea)	Flu-like symptoms are common in IL-2 related therapy	• Frequent monitoring: Patients are assessed clinically by study personnel either in person (Days 1, 2, and 3) or by phone survey (Day 4 and 5) to track progress and tolerability of flu-like symptoms, with clinical support available if necessary.
Auto-immunity	At low doses, NL-201 expands effector T-cells without significantly expanding regulatory T-cells. This combination may lead to inflammation or exacerbation of underlying autoimmune disorders	• Patient selection: This study is conducted only in patients with advanced cancer. These patients are expected to be significantly immunosuppressed at baseline due to the immunosuppressive effects of their tumors. Patients with history of clinically severe autoimmune diseases or autoimmune adverse events from previous immunotherapies are excluded from the study.
Cardiac toxicity	Minimal to mild myofiber degeneration and necrosis was noted in the highest dose of treated non-human primates in GLP toxicology experiments	• Early assessment: Patients will be required to have a recent baseline transthoracic echocardiogram and ECG prior to treatment with NL-201. • Testing: Clinical visits will include relevant safety parameters including blood pressure and pulse rate as well as questionnaire about symptoms including dizziness, syncope, and palpitations. Patients with clinical evidence of cardiovascular adverse events, including dizziness, hypotension, or syncope, will have a follow-up echocardiogram and ECG to identify potential compromise of cardiac function. • Clinical management: Depending on the severity of symptoms, patients with evidence of cardiac toxicity will be dose-reduced or receive no further doses of NL-201. Clinical support for acute cardiac toxicity can including IV fluid repletion, ionotropic support, and antiarrhythmics.
Eosinophilia	GLP toxicology experiments showed minimal to moderate elevation of peripheral eosinophils, as well as increased interleukin-5 levels	• Patient selection: Patients with hypereosinophilic syndromes or eosinophils > 2 x the ULN will be excluded. • Frequent monitoring: Patient's peripheral eosinophil counts will be assessed regularly throughout the study. • Clinical management: Eosinophilia exceeding 1.5 x 10^3 per mm^3 can be managed with dose delays, with addition of IV steroids for patients with associated symptoms, such as rash, cough or diarrhea.
Capillary leak syndrome	Systemic high dose IL-2 therapy has been known to cause capillary leak syndrome, also called vascular leak syndrome, characterized by fluid retention,	• Early assessment: Patient weights, blood chemistry, and blood pressure will be assessed on D1 of each cycle during clinical visits, allowing early identification of patients retaining fluid. • Prevention: Patients may receive pre-medication per Section 6.1.3.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
	peripheral edema, hypoalbuminemia, and hypotension.	• Clinical management: Capillary leak syndrome should be managed per Investigator discretion and according to institutional guidelines, eg, peripheral edema can be managed by dose delay, diuretic, and/or compression stockings; hypotension can be managed with IV fluids, and if necessary, vasopressors.
Hepatotoxicity	Systemic high dose IL-2 can cause hepatotoxicity due to lymphocytic infiltration of the liver sinusoids.	• Patient selection: Patients with comorbid viral hepatitis or elevation of transaminases > 3 x ULN are excluded from the study. • Early assessment: Liver function is assessed during the study, allowing for identification of hepatic toxicities.
Splenic rupture	GLP toxicology experiments showed dose-dependent reversible enlargement of the spleen and lymph nodes due to leukocyte expansion.	• Prevention: Patients will be advised to avoid high impact sports or other activities during the study
Potential risk of inflammation at the injection site	Inflammation was observed at the injection sites in the GLP toxicity studies	• Patient monitoring: Patients are dosed in a hospital setting and monitored for evidence of inflammation at the injection site and/or extravasation of NL-201. Patients with injection site inflammation that is severe enough to require intervention can be dose-reduced or withdrawn from the study. • Clinical management: In rats, inflammation of the injection site was mild to moderate and dose-dependent, while in monkeys, it was minimal to mild. In patients, mild injection site inflammation can be managed per local standard (eg, cold compresses, acetaminophen, diphenhydramine). Patients with more severe injection site inflammation can be treated with topical or systemic steroids, and/or dose reduced.
Renal toxicity	Renal tubular degeneration was observed at high doses in monkeys	• Patient selection: Patients must have eGFR $\geq$ 50 mL/min/1.73 m^2, or creatinine WNL, to participate in the study. • Early assessment: Renal function is assessed during the study, allowing for identification of renal toxicities.
Hyperviscosity	Necrosis in the bone marrow, adrenal glands and liver was observed in rats in the GLP toxicity study and was attributed to reduced perfusion/infarction associated with marked increases in circulating leukocytes	• Early assessment: The extent of leukocytosis caused by NL-201 was dose-dependent, and hematology assessments are done regularly during the study, allowing for identification and dose-reduction of patients at risk for hyperviscosity due to excessively marked leukocytosis.

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Anemia	Anemia was observed in both rats and monkeys in the GLP toxicity studies	• Patient selection: Patients with hemoglobin < 8.0 g/dL are excluded from the study. • Early assessment: Hematology assessments are done regularly during the study, allowing for identification and transfusion or dose-reduction of anemic patients.
Thrombocytopenia	Decreased platelets were observed in both rats and monkeys	• Patient selection: Patients with platelet counts < 100,000/mm^3 are excluded from the study. • Early assessment: Hematology assessments are done regularly during the study, allowing for identification and transfusion or dose-reduction of thrombocytopenic patients.
Study Procedures		
Public health related exposures during global pandemics	Completion of study procedures may increase the risk exposure to pathogens. In addition, challenges to the logistics of study procedures may arise from quarantines, site closures, travel limitations, or other considerations related to global pandemics.	If a site or region is actively affected by a global pandemic the following adjustments to the protocol will acutely apply until restrictions are removed: 1. Windows for the following visits/procedures will be expanded to the following due to operational constraints: a. Dosing visit - C2+D1 (± 3 days) (All Schedules and Parts) b. Dosing visit - CXD8 (+ 5 days) (Schedule B or step dosing) c. Radiology assessment for all visits (± 7 days) 2. Alternative methods for safety assessments (eg, phone contact, virtual visit, alternative location for assessment, including local labs or imaging centers) could be implemented when necessary and feasible. 3. Baseline PFTs are not required. 4. Vaccinations approved for the prevention of infection are allowed if completed at least 14 days before C1D1 or any time after C2D1. 5. SARS-CoV-2 testing will be required as per local regulations. 6. An enrolled patient may have a study drug (NL-201) dosing delay of up to 14 days, if the patient received an approved SARS-CoV-2 immunization or treatment before receiving their first dose of study drug on C1D1 in any schedule.

C = cycle; CD = cluster of differentiation; D = day; DMC = Data Monitoring Committee; ECG = electrocardiogram; eGFR = estimated glomerular filtration rate; GLP = Good Laboratory Practice; HIV = human immunodeficiency virus; ICU = intensive care unit; IV = intravenous; IL = interleukin; SARS-CoV-2 = severe acute respiratory coronavirus 2; PFT = pulmonary function test; ULN = upper limit of normal; WNL = within normal limits

2.3.1.1 Pembrolizumab (Parts 3 and 4 only)

Among the many immunotherapeutic strategies, immune checkpoint blockade enhances a cancer patient's immune system to fight an array of cancers. By increasing the activity of the immune system, immune checkpoint blockade can induce inflammatory side effects known as immune-related Adverse Events (irAE). Although any organ system can be affected, irAEs most commonly involve the gastrointestinal tract, endocrine glands, skin, and liver. Less often, the CNS and cardiovascular, pulmonary, musculoskeletal, and hematologic systems are involved. The wide range of potential irAEs requires multidisciplinary, collaborative management by providers across the clinical spectrum.

Immune-related adverse reactions may be mild to moderate in severity but can be severe or fatal and can occur in any organ system or tissue in patients receiving pembrolizumab and may also occur after discontinuation of treatment. For suspected immune-mediated adverse reactions, there should be adequate evaluation to confirm etiology and exclude other causes.

The key safety risks for pembrolizumab include immune-related toxicities, infusion-related reactions, and embryofetal toxicity (Table 6). Refer to Section 6.2.2.2 for specific recommendations regarding the mitigation and management of these risks.

Refer to the pembrolizumab Investigator's Brochure for additional safety information.

Table 6 Potential Risks for Pembrolizumab

Safety Risk	Description
Immune-related toxicities	Immune-related adverse effects associated with PD-1 blocking agents include pneumonitis, colitis, hepatitis, endocrinopathies, nephritis and renal dysfunction, skin reactions, encephalitis, vasculitis, sclerosing cholangitis, and other immune-related adverse reactions.
Infusion-related reactions	Signs and symptoms may include pyrexia, chills, rigors, flushing, urticaria, hypotension, dyspnea, wheezing, headache, back pain, and abdominal pain.
Embryofetal toxicity	Blockade of PD-1 signaling has been shown in animal models of pregnancy to disrupt tolerance to the fetus and to result in an increase in fetal loss; therefore, there is the possibility of fetal harm if administered during pregnancy.

PD-1 = programmed cell death 1

2.3.1.2 NL-201 Combination With Pembrolizumab (Parts 3 and 4 only)

Based on biological mechanism of action and initial clinical safety information from the monotherapy study with NL-201, the key safety risks described for monotherapy would also be expected along with the potential risks described for pembrolizumab. Moreover, with combination treatment, AEs may be more severe and occur at different time points and/or dose levels in comparison to monotherapy. In particular, target-dependent and target-independent synergistic activation of T-cells is expected and may contribute to more severe immune-related toxicities.

Potential safety risks for the combination of NL-201 and pembrolizumab include:

- Exacerbated cytokine release syndrome and/or infusion-related reactions
- Exacerbation of autoimmune/inflammatory disorders
- Exacerbation of autoimmune AEs

Patients will be monitored closely in a hospital setting for 24 hours after receiving their first dose in the first cycle. The pre-medication requirements are expected to mitigate many of the NL-201 associated toxicity.

Dose and schedule of pembrolizumab administration will be according to the approved label 200 mg every 21 days. Patients will be monitored for 30 minutes following pembrolizumab administration before NL-201 infusion is initiated. The starting dose of NL-201 in combination with pembrolizumab will be one dose level below the highest cleared dose.

Refer to the individual Investigator's Brochures for a more detailed assessment.

2.3.2 Benefit Assessment

NL-201 has been shown to effectively stimulate T-cell and NK-cell mediated antitumor activity in multiple animal models of cancer, in some cases more effectively than check-point inhibitors or IL-2. The agent is engineered to have no binding affinity for CD25, which is hypothesized to improve its safety profile. Indeed, animal safety experiments have demonstrated tolerability of NL-201 in multiple species (see Section 2.2.2), and published reports show that the parent protein Neo-2/15 is less toxic than natural IL-2.[5] An individual patient in the study may see benefit from NL-201 by experiencing stable disease (SD) or an objective response. They may potentially experience a durable CR, as has been observed with IL-2 therapy in advanced solid cancer patients. Clinical benefit in this open-label study will be estimated based on progression-free survival (PFS), ORR, and response duration.Parts 3 and 4: It is hypothesized that NL-201 in combination with pembrolizumab may improve response to pembrolizumab as was demonstrated in animal models. NL-201 has been shown to increase PD-1 expression in CD+ T-cells, increased the immune infiltration of the TME, and may make tumor cells more sensitive to the effects of pembrolizumab.

Refer to the individual Investigator's Brochures for a more detailed assessment.

2.3.3 Overall Benefit: Risk Conclusion

Patients will have advanced and incurable disease and will have exhausted all approved therapies. Under the controlled setting of clinical investigation, NL-201 offers a reasonable potential of clinical benefit. The patient will weigh the risk vs potential benefit based on informed consent.

3 OBJECTIVES AND ENDPOINTS

Parts 1 and 2 (NL-201 Monotherapy)

OBJECTIVES	ENDPOINTS
Primary	
• Assess the safety and toxicity profile of NL-201 in patients with advanced solid tumors • Define the recommended phase 2 dose (RP2D) and schedule of NL-201	• Dose-limiting toxicities (DLTs) • Incidence and severity of treatment-emergent adverse events and clinically significant changes in vital signs, electrocardiograms (ECGs), and clinical laboratory tests
Secondary	
• Characterize the pharmacokinetic (PK) profile of NL-201	• PK parameters including but not limited to area under the serum concentration time curve (AUC), maximum observed serum concentration (C_{max}), half-life ($t_{1/2}$), clearance (CL), and volume of distribution (Vd)
• Estimate the immunogenicity of NL-201	• Antidrug antibodies in serum during and after treatment with NL-201
• Estimate the antitumor activity of NL-201 per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 and RECIST for use in cancer immunotherapy trials (iRECIST) criteria	• Best objective response • Objective response rate • Duration of response • Progression-free survival (PFS)
Exploratory	
• Investigate the pharmacodynamic biomarkers of NL-201	• Flow-cytometry analysis of immune cells in blood, eg, for markers of T-cell activation and exhaustion, eg, cluster of differentiation (CD)4, CD8, CD25, forkhead box P3 (FoxP3) • Serum measurements of inflammatory cytokine levels • Analysis of immune characteristics of the tumor microenvironment (eg, multiplex immunohistochemical, transcriptional profiling)
• Estimate additional measures of antitumor activity of NL-201 per RECIST 1.1 and/or iRECIST criteria	• Overall survival (OS) • Clinical benefit rate

Parts 3 and 4 (NL-201 in Combination with Pembrolizumab)

OBJECTIVES	ENDPOINTS
Primary	
• Assess the safety and tolerability of NL-201 in combination with pembrolizumab in patients with advanced solid tumors • Define the RP2D and schedule of NL-201 in combination with pembrolizumab	• DLTs • Incidence and severity of treatment-emergent adverse events and clinically significant changes in vital signs, ECGs, and clinical laboratory tests
Secondary	
• Characterize the PK profile of NL-201 in combination with pembrolizumab	• NL-201 PK parameters including but not limited to AUC, C_{max}, $t_{1/2}$, CL, and Vd

OBJECTIVES	ENDPOINTS
• Estimate the immunogenicity of NL-201 in combination with pembrolizumab	• NL-201 antidrug antibodies in serum during and after treatment with NL-201 in combination with pembrolizumab
• Estimate the antitumor activity of NL-201 in combination with pembrolizumab per RECIST 1.1 and iRECIST criteria	• Best objective response • Objective response rate • Duration of response • PFS
Exploratory	
• Investigate the pharmacodynamic biomarkers of NL-201 in combination with pembrolizumab	• Flow-cytometry analysis of immune cells in blood for modulation of lymphocyte subsets, including markers of T-cell activation and exhaustion, including CD4, CD8, CD25, FoxP3 • Serum measurements of inflammatory cytokine levels • Analysis of immune characteristics of the tumor microenvironment (eg, multiplex immunohistochemical, transcriptional profiling)
• Evaluation of exploratory biomarkers including pharmacodynamic and potential patient selection biomarkers	• PD-L1 expression in tumors (as available) • Tumor microenvironment (TME) immune score (as available)
• Estimate additional measures of antitumor activity of NL-201 in combination with pembrolizumab per RECIST 1.1 and/or iRECIST criteria	• OS • Clinical benefit rate

4 STUDY DESIGN

4.1 Overall Design

This is a Phase 1 first-in-human, open-label, dose escalation and cohort-expansion study of NL-201 that will be conducted in 4 parts.

Parts 1 and 2 (NL-201 Monotherapy)

Part 1 will be an adaptive modified toxicity probability interval (mTPI) design dose escalation study in adult patients with advanced solid tumors to determine safety profile and the recommended Phase 2 dose (RP2D) and treatment schedule of NL-201 as monotherapy. Part 1 will include IV (Schedule A and B), each with up to approximately 50 patients In addition, there will be backfill cohorts (up to approximately 12 patients in total) , at certain DMC-cleared dose levels and schedules, to collect, PK, PD and response data in certain tumor types or to explore additional pre-medication regimens (eg, steroids). The decision to enroll patients into backfill cohorts and to define the patients to be enrolled will be made by the Sponsor. Part 1 will enroll up to approximately 115 adult patients in total.

Part 2 will be a Simon 2-stage dose expansion cohort study in patients in 2 indications (up to n = 30 per cohort) with exploratory paired biopsies to estimate the tolerability and antitumor activity of NL-201 as monotherapy in these indications.

Parts 3 and 4 (NL-201 in Combination with Pembrolizumab)

Part 3 will be an adaptive mTPI design dose escalation study in up to 42 adult patients with advanced solid tumors to determine the safety profile, RP2D and treatment schedule of NL-201 in combination with pembrolizumab. Part 4 will be a dose expansion phase in 3 cohorts (up to n = 30 per cohort) to confirm the combination RP2D and obtain additional estimation of pharmacokinetics (PK),pharmacodynamics (PDn), and antitumor activity.

All Parts

Patients will enter the screening period, up to 28 days prior to Cycle 1 Day 1 of treatment. PK, PDn, and exploratory tumor biopsy samples will be obtained per the study Schedule of Events (see Section 1.3). Tumor response to treatment will be assessed by radiographic evaluation per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 and RECIST for use in cancer immunotherapy trials (iRECIST) every 6 weeks for 12 weeks, and every 12 weeks thereafter while on treatment. Patients may continue on treatment until unacceptable toxicity, disease progression per iRECIST with either confirmation or clinical decline, completion of 35 administrations (approximately 2 years) with pembrolizumab (for Parts 3 and 4), withdrawal of consent, patient or physician decision, study termination, or death. Patients with progressive disease who are considered to be clinically stable and whose progression is considered to be minimal are required to have a confirmatory scan and tumor evaluation after the initial determination of progression. Patients with progressive disease per RECIST criteria may continue on treatment past progression if, in the opinion of the Investigator, the patient is considered to be clinically stable or has experienced clinical benefit from NL-201 alone or in combination with pembrolizumab (see Section 6.1.1.1).

Upon permanent discontinuation of study drug for any reason, an end of treatment visit should be scheduled as soon as possible, but within 30 days following cessation of study treatment or before patient initiates new anti-cancer therapy, whichever is earlier. All AEs and concomitant medication will be followed up to 30 days after last dose of NL-201.

For Parts 3 and 4, all adverse events will be followed up to 30 days following cessation of study treatment and all serious adverse events will be followed up to 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anti-cancer therapy, whichever is earlier. Patients who discontinue from treatment due to progressive disease based on RECIST or iRECIST will enter long-term follow-up to assess survival and subsequent anti-cancer therapies. Patients in Parts 2 and 4 will also need to record any subsequent anti-cancer therapies during long-term follow-up.

For Parts 2 and 4, patients who discontinue from treatment due to reasons other than progressive disease based on RECIST or iRECIST will be followed in radiologic follow-up for documented progressive disease by RECIST 1.1 and will enter long-term follow-up. If subsequent anti-cancer therapies are initiated during the period of time between discontinuation of treatment and progressive disease documentation, the patient will no longer be radiologically assessed for progression and will enter long-term follow-up.

Patients in long-term follow-up will be followed until death, withdrawal of consent, lost to follow-up, or study closure, whichever occurs first, for a maximum of 2 years after the end of treatment.

Part 1: Intrasubject Dose Escalation

Intrasubject dose escalation is permitted with Medical Monitor approval. In cases where no treatment-related Grade 2 or higher adverse events have been observed, dose escalation to a higher dose will be permitted provided that the dose level has been cleared by the DMC.

Part 1: Optional Tumor Biopsy

During dose escalation, paired biopsies are optional. For patients who consent to have paired biopsies, the same lesion will be sampled within 28 days prior to the first dose of NL-201 (ie, pre-treatment biopsy) and on study (ie, post-treatment biopsy). The post-treatment, second biopsy will occur between 5 to 9 days after receiving dose at Cycle 2 Day 1 and for Schedule B patients, before the dosing at Cycle 2 Day 8 visit. The lesion to be biopsied cannot be the only site of measurable disease.

Part 1: IV Dose Escalation Study of NL-201

Dose escalation will follow a mTPI design[25] to guide dose escalation and de-escalation within each schedule. NL-201 will be administered as an IV infusion in 21-day cycles in cohorts of $n \geq 3$ patients at a time. Schedule A cohorts will be dosed every 21 days, while Schedule B cohorts will be dosed on Days 1 and 8 of every 21-day cycle.

The Schedule A and Schedule B cohorts will enroll on a rolling basis in parallel and cohorts will be managed by a Cohort Management Plan. The first new patient of each new dose level (for both Schedule A and Schedule B) shall be observed for 48 hours after their first infusion prior to enrolling subsequent patients to that dose level and schedule.

In addition, there will be backfill cohorts (up to approximately 12 patients in total) , at certain DMC-cleared dose levels and schedules, to collect, pharmacokinetic, pharmacodynamic and response data in certain tumor types or to explore additional pre-medication regimens (eg. steroids). These patients will not be part of the DLT evaluation but will be included in the safety population.

Refer to Section 6.2.1 for details regarding dose escalation. The final decision in terms of the dose to be applied to the next set of $n \geq 3$ patients will be made by the DMC based upon the mTPI design together with the available safety and laboratory data.

Based on the totality of data arising in Part 1, the DMC will determine a RP2D. If the RP2D requires step-dosing, the step-dosing regimen would be incorporated into dosing for patients in Part 2.

Part 2: Monotherapy Dose Expansion Study of NL-201

This monotherapy dose expansion cohort study will evaluate safety and estimate antitumor activity of NL-201 as monotherapy in diseases that are responsive to IL-2, ie, (1) malignant melanoma and (2) RCC. The RP2D (and treatment schedule) for these cohorts will be determined in Part 1.

Expansion cohorts will individually comprise up to 30 patients each, of which a minimum of 8 patients per cohort will be required to have paired biopsies of the same lesion before and after treatment with NL-201. Pre-treatment biopsies will occur up to 28 days prior to the first dose (Cycle 1 Day 1), while post-treatment biopsies will occur 7 (± 2) days after the Cycle 2 dose.

Part 3: Dose Escalation Study of NL-201 in Combination with Pembrolizumab

Dose escalation will follow a mTPI design to guide dose escalation and de-escalation within each schedule. NL-201 will be administered as an IV infusion in 21-day cycles in cohorts of $n \geq 3$ patients at a time. The starting dose of NL-201 will be at least 1 dose level below the highest dose level that has been recommended as safe and tolerable by DMC from Part 1 of the study.

Schedule A cohorts will be dosed every 21 days, while Schedule B cohorts will be dosed on Days 1 and 8 of every 21-day cycle. A fixed dose of 200 mg pembrolizumab will be administered on Day 1 of every 21-day cycle, regardless of Schedule. Pembrolizumab will be administered before NL-201. NL-201 infusion will start 30-60 minutes after pembrolizumab infusion is complete. Refer to Section 6.1.1 for additional details regarding drug administration. The DMC may modify the NL-201 and pembrolizumab dosing schedule based on emerging safety data.

Schedule A will open first to enrollment. Schedule B can be started subsequently as determined by safety from monotherapy, provided that each dose level must be cleared in Schedule A before enrolling to Schedule B at the same dose level. If Schedule A and Schedule B are enrolling simultaneously, Schedule A enrollment will be prioritized over Schedule B to ensure no delay to study enrollment. The first new patient of each new dose level (for both Schedule A and Schedule B) shall be observed for 96 hours after the conclusion of their infusion(s), prior to enrolling subsequent patients to that dose level and schedule.

The final decision in terms of the dose to be applied to the next set of $n \geq 3$ patients will be made by the DMC based upon the mTPI design together with the available safety and laboratory data.

Based on the totality of data arising in Part 3, the DMC will determine a RP2D for NL-201 in combination with pembrolizumab. If the combination RP2D requires step-dosing, the step-dosing regimen would be incorporated into dosing for patients in Part 4.

Part 4: Dose Expansion Study of NL-201 in Combination with Pembrolizumab

This combination therapy dose expansion cohort will further evaluate safety and estimate antitumor activity of NL-201 in combination with pembrolizumab at the selected RP2D (dose

and schedule) determined in Part 3 in diseases that are responsive to IL-2, ie, (1) immune checkpoint inhibitors (ICI) naïve malignant melanoma, (2) ICI naïve RCC, and (3) patients with non-small cell lung cancer (NSCLC), melanoma, or head and neck squamous cell carcinoma (HNSCC) who have been treated with pembrolizumab achieving a best response of SD.

Expansion cohorts will individually comprise up to 30 patients each, of which a minimum of 8 patients per cohort will be required to have paired biopsies of the same lesion before and after treatment with NL-201. Pre-treatment biopsies will occur up to 28 days prior to the first dose (Cycle 1 Day 1), while post-treatment biopsies will occur 7 (± 2) days after the Cycle 2 dose.

4.1.1 Study Duration for Patients

The duration of screening is up to 28 days prior to Cycle 1 Day 1 and the duration of treatment for an individual patient is anticipated to be a median of 3 months. For Parts 2 and 4 only, this will be followed by radiology follow-up visits until disease progression or initiation of new therapy. Upon disease progression or initiation of new therapy, patients enter long term follow-up (every 6 months ± 1 month) until death, lost to follow-up, withdrawal of consent or study closure, whichever occurs first, for a maximum of 2 years after end of treatment. Long term follow-up visits may occur over the telephone.

The total study duration for an individual patient is estimated to be approximately 12 months.

4.1.2 Number of Patients

Part 1 will enroll up to approximately 50 patients for each of IV Schedule A, IV Schedule B in cohorts of $n \geq 3$ for dose escalation, and up to approximately 12 patients in total for backfill cohorts at certain dose levels and schedules which have been previously cleared by DMC In total, up to approximately 115 patients will be enrolled in Part 1.

Part 2 will enroll up to 30 patients in each cohort, up to a maximum of 60 patients in total. Part 3 will enroll up to 21 patients for each of Schedule A and Schedule B in cohorts of $n \geq 3$ for a maximum of 42 patients in total. Part 4 will enroll up to 30 patients in each cohort, up to a maximum of 90 patients in total.

Combined enrollment for all parts of the study is up to an approximately 310 patients.

4.1.3 Replacement of Patients

In Parts 1 and 3, patients who are unable to be evaluated for toxicity during the DLT window due to discontinuation for unrelated AEs or disease progression will be replaced. In Parts 2 and 4, patients who are withdrawn or removed from treatment or the study will not be replaced.

4.1.4 Number of Sites

Approximately 10 sites in North America and Australia will participate in Parts 1 and 3, and approximately 20 sites in North America and Australia will participate in Parts 2 and 4 of this

global study. During the conduct of the study, additional sites and countries within these regions may be added as necessary.

4.2 Scientific Rationale for Study Design

Aldesleukin is a recombinant human IL-2 protein that has monotherapeutic efficacy in malignant melanoma and RCC. It activates T-cell-mediated tumor inhibition by heterodimerizing the beta and gamma subunits of the IL-2 receptor, leading to phosphorylation of STAT5. However, serious toxicity has limited the clinical utility of IL-2.[26] The cause of this toxicity is related to its high binding affinity for CD25+ cells: preferential activation of CD25+ cells, such as endothelial cells and regulatory T-cells, leads to vascular leak and immunosuppression.[27,28] NL-201 is a novel immunotherapeutic based on a de novo designed mimic of IL-2 that acts through the same mechanism of action as IL-2 but has no affinity for CD25. The parental protein of NL-201 binds with high affinity and in the correct orientation to the beta and gamma subunits of the IL-2 receptor without any affinity for CD25, and leads to significantly less toxicity than recombinant IL-2 in animal models.[5] NL-201 triggers STAT5 phosphorylation with high potency, and leads to inflammatory cytokine signaling, tumor inhibition and prolonged survival in animal models. These studies suggest that NL-201 may provide antitumor benefit with an improved safety profile.

4.3 Justification for Dose

Part 1: NL-201 IV Monotherapy Dose Escalation

The FIH starting dose was selected based on evaluation of the totality of the nonclinical data including *in vitro* evaluation of PDn effects in human peripheral blood mononuclear cells (PBMCs), the most sensitive assay, and *in vivo* toxicity data in monkeys, the most sensitive species. The proposed starting dose of 0.1 µg/kg is predicted to result in an *in vivo* C_{max} in patients of approximately 2 ng/mL. The proposed dose is anticipated to approximate the lowest pharmacologically active dose (PAD) based on *in vitro* PDn evaluations and is 150- to 300-fold below the C_{max} achieved in monkeys given 11.44 µg/kg NL-201; a dose considered to be the NOAEL (see Table 8 for dose escalation scheme).

Part 2: NL-201 Monotherapy Dose Expansion

The recommended monotherapy dose and schedule of NL-201 that is determined during dose escalation will be used during monotherapy dose expansion (Part 1). The DMC will determine this dose and schedule by considering the PK, PDn, tolerability, antitumor activity, and the occurrence of any cumulative toxicities over multiple cycles of NL-201. No randomization or dose escalation will occur, though dose reduction or delay for toxicity will be allowed.

Part 3: NL-201 in Combination with Pembrolizumab Dose Escalation

The planned dose of pembrolizumab for this study is 200 mg every 3 weeks (Q3W). Based on the totality of data generated in the Keytruda development program, 200 mg Q3W is an appropriate dose of pembrolizumab for adults across all indications. As outlined below, this dose is justified by:

- Clinical data from 8 randomized studies in melanoma and NSCLC indications demonstrating flat dose- and exposure-efficacy relationships from 2 mg/kg Q3W to 10 mg/kg every 2 weeks (Q2W) representing an approximate 5- to 7.5-fold exposure range (refer to the Investigator's Brochure)
- Population PK analysis showing that both fixed dosing and weight-based dosing provides similar control of PK variability with considerable overlap in the distributions of exposures, supporting suitability of 200 mg Q3W
- Clinical data showing meaningful improvement in benefit-risk including overall survival (OS) at 200 mg Q3W across multiple indications, and
- Pharmacology data showing full target saturation in both systemic circulation (inferred from PK data) and tumor (inferred from physiologically-based PK [PBPK] analysis) at 200 mg Q3W.

Among the 8 randomized dose-comparison studies, a total of 2262 participants were enrolled with melanoma and NSCLC, covering different disease settings (treatment naïve, previously treated, PD-L1 enriched, and all-comers) and different treatment settings (monotherapy and in combination with chemotherapy). Five studies compared 2 mg/kg Q3W versus 10 mg/kg Q2W (KN001 Cohort B2, KN001 Cohort D, KN002, KN010, and KN021), and 3 studies compared 10 mg/kg Q3W versus 10 mg/kg Q2W (KN001 Cohort B3, KN001 Cohort F2 and KN006). All of these studies demonstrated flat dose- and exposure-response relationships across the doses studied representing an approximate 5- to 7.5-fold difference in exposure. The 2 mg/kg (or 200 mg fixed-dose) Q3W provided similar responses to the highest doses studied. Subsequently, flat dose-exposure-response relationships were also observed in other tumor types including head and neck cancer, bladder cancer, gastric cancer and classical Hodgkin Lymphoma, confirming 200 mg Q3W as the appropriate dose independent of the tumor type. These findings are consistent with the mechanism of action of pembrolizumab, which acts by interaction with immune cells, and not via direct binding to cancer cells.

Additionally, pharmacology data clearly show target saturation at 200 mg Q3W. First, PK data in KN001 evaluating target-mediated drug disposition (TMDD) conclusively demonstrated saturation of PD-1 in systemic circulation at doses much lower than 200 mg Q3W. Second, a PBPK analysis was conducted to predict tumor PD-1 saturation over a wide range of tumor penetration and PD-1 expression. This evaluation concluded that pembrolizumab at 200 mg Q3W achieves full PD-1 saturation in both blood and tumor.

Finally, population PK analysis of pembrolizumab, which characterized the influence of body weight and other participant covariates on exposure, has shown that the fixed-dosing provides similar control of PK variability as weight-based dosing, with considerable overlap in the distribution of exposures from the 200 mg Q3W fixed dose and 2 mg/kg Q3W dose. Supported by these PK characteristics and given that fixed-dose has advantages of reduced dosing complexity and reduced potential of dosing errors, the 200 mg Q3W fixed-dose was selected for evaluation across all pembrolizumab protocols.

Part 4: NL-201 in Combination with Pembrolizumab Dose Expansion

The recommended dose and schedule of NL-201 in combination with pembrolizumab that is determined during dose escalation (Part 3) will be used during combination dose expansion. Supported by the mTPI design, the DMC will determine this dose and schedule by considering the PK, PDn, and any cumulative toxicities over multiple cycles of NL-201 in combination with pembrolizumab. No randomization or dose escalation will occur, though dose reduction or delay for toxicity will be allowed.

4.4 End of Study

End of Study (Individual Patient): A patient is considered to have completed the study if he/she has completed all phases of the study including the last visit shown in the Schedule of Events (Section 1.3; eg, last long-term follow up visit as described in Section 0).

Primary Completion: The primary completion date is defined as the date when the last patient is assessed or receives an intervention for the final collection of data to support the primary endpoint(s) for the purposes of conducting the primary analysis. This date is defined to be up to 30 days after the last patient has completed the last treatment.

End of Study: The end of the study is defined as the date of the last visit of the last patient in the study or last scheduled procedure shown in the Schedule of Events (Section 1.3) for the last patient in the study globally. The total study duration, including all follow-up, is expected to be approximately 5 years.

4.5 Patient Input on Study Design

Patient input was not obtained for this study.

5 STUDY POPULATION

Before any study-specific activities/procedures, the appropriate written informed consent must be obtained (see Section 10.1.3). Patients must meet all enrollment criteria to be eligible for this study.

Part 1 target population: Adult patients with measurable advanced and incurable solid tumors who have failed all lines of treatment, including CPIs if indicated.

Part 2 target population: Adult patients with pathologically proven diagnosis of the target disease indications, (1) malignant melanoma and (2) RCC, who have advanced and incurable disease and have failed at least 1 line of treatment, including CPIs.

Part 3 target population: Adult patients with histologically-confirmed solid tumors who have received ≥ 1 prior line of therapy for advanced or metastatic disease.

Part 4 target population: Adult patients with pathologically proven diagnosis of the target disease indications, (1) ICI naïve malignant melanoma and (2) ICI naïve RCC, and (3) patients with

NSCLC, melanoma, or HNSCC who have received prior treatment with pembrolizumab achieving a best response of SD.

5.1 Inclusion Criteria

Patients are eligible to be included in the study only if all of the following criteria apply at the time of screening:

1. Patients ≥ 18 years of age inclusive, at the time of signing the informed consent.
2. Capable of giving informed consent as described in Section 10.1.3, which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.
3. Patients with measurable disease per RECIST 1.1 as assessed by the local site investigator/radiology. Lesions situated in a previously irradiated area are considered measurable if progression has been demonstrated in such lesions.
4. Patients with Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.
5. Patients with adequate organ function, defined as:
   a. Hematologic function laboratory values (specimens must be collected within 10 days prior to the start of study treatment):
      i. Platelets ≥ 100,000/mm^3
      ii. Absolute neutrophil count (ANC) > 1500/mm^3
      iii. Hemoglobin: Parts 1 and 2: ≥ 8.0 g/dL; Parts 3 and 4: ≥ 9.0 g/dL (NOTE: Criteria must be met without packed red blood cell transfusion within the prior 2 weeks. Participants can be on stable dose of erythropoietin (≥ approximately 3 months).
      iv. Lymphocyte count ≥ 500/mm^3
      v. Eosinophils < 1000/mm^3
   b. Renal function (specimens must be collected within 10 days prior to the start of study treatment): estimated glomerular filtration rate (eGFR) ≥ 50 mL/min/1.73 m^2 as determined by 2009 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation,[29] or creatinine within normal limits (WNL)
   c. Hepatic function (specimens must be collected within 10 days prior to the start of study treatment):
      i. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT): Parts 1 and 2: ≤ 3 x upper limit of normal (ULN), or ≤ 5 x ULN if known liver malignancy or metastasis; Parts 3 and 4: AST/ALT ≤ 2.5 x ULN, or ≤ 5 x ULN if known liver malignancy or metastasis
      ii. Total bilirubin (TBL) WNL, or ≤ 3 x ULN if known Gilbert's syndrome
   d. Adequate cardiovascular and respiratory function with transthoracic echocardiogram (ECHO) and pulmonary function testing showing no clinically significant findings. If ECHO is not available or appropriate according to the institutional standard, then multi-gated acquisition (MUGA) scan is permitted.

e. Coagulation (specimens must be collected within 10 days prior to the start of study treatment): international normalized ratio (INR) OR prothrombin time (PT) ≤ 1.5 x ULN unless participant is receiving anticoagulant therapy as long as PT or partial thromboplastin time (aPTT) is within therapeutic range of intended use of anticoagulants

6. At least 6 weeks from any prior nitrosurea or mitomycin C therapy; at least 4 weeks from any other prior chemotherapy or CPI; at least 2 weeks from any kinase inhibitor.
7. Women are not pregnant or breastfeeding and 1 of the following conditions applies:
   a. Is a woman of non-childbearing potential as defined in Section 10.4
   b. Is a woman of childbearing potential (WOCBP) and using a contraceptive method that is highly effective, with a failure rate of < 1% (as described in Section 10.4) during the study treatment period and for at least 60 days after the last dose of NL-201 (Parts 3 and 4 only: 120 days after the last dose of study treatment). The Investigator should evaluate the potential for contraceptive method failure (eg, noncompliance, recently initiated) in relationship to the first dose of study drug.
8. A WOCBP must have a negative highly sensitive pregnancy test (serum or urine) at screening.
9. Men are eligible to participate if they agree to the following during the study drug period and for at least 90 days after the last dose of NL-201 (Parts 3 and 4 only: 120 days after the last dose of pembrolizumab):
   a. Refrain from donating sperm
   b. For men with partners of childbearing potential or partners who are pregnant or breastfeeding: Use a male condom and should also be advised of the benefit for a female partner to use a highly effective method of contraception as a condom may break or leak when having sexual intercourse with a woman of childbearing potential who is not currently pregnant.

Part 1 Only

10. Patients with any relapsed or refractory advanced solid tumor, other than prostate cancer, who have progressed, not tolerated or are ineligible for all approved non-IL-2-based lines of therapy.

Parts 2 and 4 Only

11. Patients with a diagnosed target disease indication that has failed at least 1 line of systemic therapy.

Parts 3 and 4 Only

12. Part 3 only: Patients with histologically-confirmed solid tumors who have received ≥ 1 prior line of therapy for advanced or metastatic disease.
13. Part 4 only: Patients with a diagnosed target disease indication OR have received prior treatment with pembrolizumab achieving a best response of SD.
14. Part 4 only: Have archival tumor tissue sample available or newly obtained core/excisional biopsy of a tumor lesion not previously irradiated. Formalin-fixed, paraffin embedded (FFPE) tissue blocks are preferred to slides. If archival tissue is not available, and there is not a readily accessible lesion for biopsy, the biopsy may be waived with Sponsor approval.

Note: If submitting unstained cut slides, newly cut slides should be submitted to the testing laboratory within 14 days from the date slides are cut (details pertaining to tumor tissue submission can be found in the Procedures Manual).

Note: Archival biopsy should be within 5 years of screen date for PD-L1 analysis.

5.2 Exclusion Criteria

Patients are excluded from the study if any of the following criteria apply:

Medical Conditions

1. Known active CNS metastases and/or carcinomatous meningitis. Participants with previously treated brain metastases may participate provided they are radiologically stable, ie, without evidence of progression for at least 4 weeks by repeat imaging (note that the repeat imaging should be performed during study screening), clinically stable and without requirement of steroid treatment for at least 14 days prior to first dose of study treatment.
2. Development of clinically severe non-endocrine autoimmune disorder leading to discontinuation of prior immunotherapy (eg, pneumonitis, myocarditis, neuropathy, or nephritis).
3. Active autoimmune disease that has required systemic treatment in past 2 years (ie, with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg, thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency) is not considered a form of systemic treatment and is allowed.
4. History of solid organ transplant or bone marrow transplant.
5. Myocardial infarction in the 6 months prior to initiating therapy.
6. Class II or greater heart failure per New York Heart Association Classification.
7. Clinically significant bleeding, such as gastrointestinal or intracranial bleeding, or deep vein thrombosis or pulmonary embolism in the 3 months prior to initiating therapy. Thrombus prophylaxis is acceptable.
8. Evidence or clinical suspicion of ongoing serious active infection requiring systemic treatment.
9. Known history of human immunodeficiency virus (HIV) or positive for HIV at screening.
10. Known chronic or active viral hepatitis.
11. Known or suspected severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, unless patient tests negative for SARS-CoV-2 within the screening period.
12. Parts 3 and 4 only: Has a history of (non-infectious) pneumonitis/interstitial lung disease that required steroids or has current pneumonitis/interstitial lung disease.
13. Parts 3 and 4 only: Has a known additional malignancy that is progressing or has required active treatment within the past 2 years.

    Note: Participants with basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or carcinoma in situ (eg, breast carcinoma, cervical cancer in situ) that have undergone potentially curative therapy are not excluded.

Prior/Concomitant Therapy

14. Live or live-attenuated vaccine(s) within 30 days prior to screening or plans to receive such vaccines during the study. Note: Administration of killed vaccines are allowed.
15. Prior chimeric antigen receptor T-cell (CAR-T) or allogeneic cellular therapy.
16. Prior IL-2-based cancer immunotherapy.
17. Diagnosis of immunodeficiency or is receiving ongoing systemic immunosuppressive therapy (stable replacement doses of systemic prednisone ≤ 10 mg/day or equivalent will be allowed) or any other form of immunosuppressive therapy within 7 days prior the first dose of study treatment.
18. History of hypersensitivity to PEG or PEGylated drugs.
19. Parts 3 and 4 only: Has severe hypersensitivity (≥ Grade 3) to pembrolizumab and/or any of its excipients.
20. Parts 3 and 4 only: Patients on a prior PD-1 or PD-L1 inhibitor who experienced a Grade 3 or higher irAEs.
21. Parts 3 and 4 only: Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti PD-L2 agent or with an agent directed to another stimulatory or co-inhibitory T-cell receptor (eg, CTLA-4, OX 40, CD137), and was discontinued from that treatment due to a Grade 3 or higher irAE.
22. Parts 3 and 4 only: Has received prior systemic anti-cancer therapy including investigational agents within 4 weeks (could consider shorter interval for kinase inhibitors or other short half-life drugs) prior to treatment.

    Note: Participants must have recovered from all AEs due to previous therapies to ≤ Grade 1 or baseline. Participants with ≤ Grade 2 neuropathy may be eligible. Participants with endocrine-related AEs Grade ≤ 2 requiring treatment or hormone replacement may be eligible.

    Note: If the participant had major surgery, the participant must have recovered adequately from the procedure and/or any complications from the surgery prior to starting study intervention.
23. Parts 3 and 4 only: Has received prior radiotherapy within 2 weeks of start of study treatment. Participants must have recovered from all radiation-related toxicities, not require corticosteroids, and not have had radiation pneumonitis. A 1-week washout is permitted for palliative radiation (≤ 2 weeks of radiotherapy) to non-CNS disease.
24. History of drug-induced Stevens Johnson syndrome, drug reaction with eosinophilia and systemic symptoms (DRESS syndrome) or drug induced toxic epidermal necrolysis.
25. Anticoagulation, unless patient has been on a stable dose of their anticoagulant for ≥ 4 weeks without new thrombotic or clinically significant bleeding events.
26. Concurrent therapy with any other investigational agent, vaccine, or device. Concomitant participation in observational studies is acceptable after Sponsor approval.

Prior/Concurrent Clinical Study Experience

27. Current enrollment or past participation within the last 28 days before first planned dose (Cycle 1 Day 1) in any other clinical study involving an investigational study treatment or any other type of medical research.

    Note: Participants who have entered the follow-up phase of an investigational study may participate as long as it has been 4 weeks after the last dose of the previous investigational agent.

Diagnostic Assessments

28. Diabetes mellitus with hemoglobin A1c ≥ 8.5% at screening.
29. Corrected QT interval (QTc) > 470 milliseconds or known history of familial long QT syndrome.

Other Exclusions

30. Major surgery in the 8 weeks prior to initiating therapy.
31. Any serious medical condition (including preexisting autoimmune disease or inflammatory disorder), laboratory abnormality, psychiatric condition, or any other significant or unstable concurrent medical illness that in the opinion of the Investigator would preclude protocol adherence or would make the safety of the study drug difficult to assess.

5.3 Screen Failures

Screen failures are defined as patients who consent to participate in the clinical study but do not meet inclusion/exclusion criteria for study treatment. A minimal set of screen failure information is required to ensure transparent reporting of screen failure patients to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened 1 time. Informed consent is required before patients can be rescreened if outside the original 28-day screening window. See Section 10.1.3 for Informed Consent Process details.

6 STUDY TREATMENT

Study drug is defined as any investigational intervention(s), non-investigational product(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study patient according to the study protocol.

Refer to the Pharmacy Instructions for more detailed information regarding the storage, preparation, destruction, and administration of each treatment.

6.1 Study Drug(s) Administered

6.1.1 Investigational Products

Table 7 Investigational Products: NL-201 and Pembrolizumab

Intervention Name	NL-201	Pembrolizumab (Parts 3 and 4 only)
Type	Biologic	
Dose Formulation	Solution for infusion	Solution for infusion
Unit Dose Strength(s)	See Table 8 and Table 9	100 mg/vial
Dosage Level(s)		200 mg Q3W
Route of Administration	IV infusion	IV infusion
Dosing Instructions	NL-201 will be dosed as per the Pharmacy Instructions. IV dosing will occur either every 21 days (for Schedule A) or on Days 1 and 8 of every 21-day cycle (for Schedule B). For IV: patients that weigh ≥ 100 kg, the dose is capped as to not allow more than 100 times the current dose level to be administered to a patient at 1 time (see Table 8 and Table 9). Parts 3 and 4 only: NL-201 to be administered 30-60 minutes post administration of pembrolizumab.	Pembrolizumab will be administered using IV infusion on Day 1 of each 21-day cycle after all procedures and assessments have been completed. Pembrolizumab will be administered as a dose of 200 mg using a 30-minute IV infusion. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window between -5 minutes and +10 minutes is permitted (ie, infusion time is 30 minutes (-5 min/+10 min). After Cycle 1 trial treatment of pembrolizumab may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons, however, pembrolizumab must always be administered on the same day as NL-201 when used in combination. The Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion and administration of infusion solution.
Hospitalization Guidance	Patients will remain in a hospital setting for the first 24 hours after completion of their first dose (IV) on study (may be extended per Investigator discretion), and for 8 hours after receiving subsequent doses. This period of hospital observation will be reduced to 2 hours for patients that have tolerated 3 consecutive doses. Required monitoring times may be adjusted by Neoleukin and/or per DMC recommendation pending review of safety data. Patients should also be advised to stay within a reasonable distance of the clinical site for the first week after the first dose.	
Use	Investigational	
Sourcing	Provided centrally by the Sponsor.	
Packaging and Labeling	Study drug will be provided in glass vials. Each glass vial will be labeled as required per country requirement.	

DMC = Data Monitoring Committee; IV = intravenous; Q3W = every 3 weeks

NOTE: Refer to the Pharmacy Instructions for further details.

NL-201 will be administered until unacceptable toxicity, disease progression, withdrawal of consent, patient or physician decision, or study termination. Patients with progressive disease who have experienced clinical benefit from NL-201 will have the option to continue on therapy past progression.

6.1.1.1 Treatment Beyond Disease Progression

Because of the possibility of an initial increase in tumor burden caused by immune cell infiltration in the setting of a T-cell response (termed pseudoprogression), radiographic progression per RECIST 1.1 may not be indicative of true disease progression. The iRECIST criteria allows for continued treatment beyond apparent progression of disease in order to confirm response. In patients who have initial evidence of radiological disease progression per RECIST 1.1, it is at the discretion of the treating physician whether to continue a patient on study treatment until repeat imaging is obtained. This clinical judgement decision should be based on the patient's overall clinical condition, including performance status, clinical symptoms, and laboratory data. Patient may receive study treatment while waiting for confirmation of disease progression if they are clinically stable as defined by the following:

- Evidence of clinical benefit as assessed by the Investigator (without any rapid disease progression)
- Tolerance of NL-201
- No decline in ECOG performance status that can be attributed to disease progression
- Absence of tumor progression at critical anatomical sites (eg, leptomeningeal disease) that cannot be managed by protocol-allowed medical interventions
- Treatment beyond progression will not delay an imminent intervention to prevent serious complications of disease progression (eg, no evidence that disease progression may result in impending organ impairment)

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

Patients who continue treatment beyond radiographic disease progression per RECIST 1.1 should be closely monitored clinically and with a follow-up scan in 4 weeks or sooner at the Investigator’s discretion. Treatment should be discontinued if clinical deterioration due to disease progression occurs at any time, or if persistent disease growth is confirmed in a follow-up scan. In addition, patients should be discontinued for unacceptable toxicity or for any other signs or symptoms of deterioration attributed to disease progression as determined by the Investigator after an integrated assessment of radiographic data and clinical status.

6.1.2 Preparation/Handling/Storage/Accountability

- The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit and storage for all study drug received and any discrepancies are reported and resolved before use of the study drug.
- Only patients enrolled in the study may receive study drug and only authorized site staff may supply or administer study drug. All study drug must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff.
- The same lot should be used intra-patient.
- The Investigator, institution, or the head of the medical institution (where applicable) is responsible for study drug accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).
- Further guidance and information for the final disposition of unused study drugs are provided in the Pharmacy Instructions.

6.1.3 Other Protocol-Required Intervention

To mitigate potential NL-201-related, cytokine associated toxicities, pre-treatment is required to be administered before the start of each NL-201 dose for:

- NL-201 IV doses ≥ 0.6 μg/kg monotherapy,
- NL-201 doses in combination with pembrolizumab

Required Pre-Medication Protocol for NL-201:

1) Pre-medication Requirement: Administration of an agent from each of the following two therapeutic classes must have been completed within 30 to 60 minutes before the start of NL-201 administration:
   a) Anti-pyretic agent (325-1000 mg acetaminophen [or equivalent]) and to follow NL-201 administration with ongoing anti-pyretic as needed to control fever.
   b) Histamine receptor antagonist (50-100 mg diphenhydramine [or equivalent]).

The specific drugs discussed above should be selected according to the institutional guidelines. Cases where a patient has a medical condition that precludes pretreatment should be discussed with the Medical Monitor.

- Hydration Requirement: Unless medically contraindicated, infusion of at least 1 liter of normal saline completed on the day of each NL-201 dose administration.
  a) It is recommended that patients be instructed to orally hydrate with at least 1 liter of appropriate fluids per day for at least 2 days after each NL-201 dose.
- Patients should hold all antihypertensive medications for 12 to 48 hours prior to the administration of NL-201 unless medically contraindicated.

Although continued pre-medication is recommended for all NL-201 administrations, after Cycle 2, the investigator may adjust the dosage and/or avoid using certain pre-medication agents during the treatment, if supported by the investigator's assessment of the risk from cytokine release syndrome, capillary leak syndrome or other infusion-related reactions.

Pre-medication is also permitted if a patient experiences signs or symptoms of cytokine release syndrome, capillary leak syndrome or other infusion-related reaction at lower doses. The DMC may recommend further exploration with additional toxicity mitigations, such as steroids.

For patients that require pre-treatment with antihistamine and/or IV fluids for pembrolizumab, dosing of antihistamine should not be repeated before NL-201 and IV fluids should only be given prior to pembrolizumab, but as close to the start of pembrolizumab infusion as possible.

6.1.4 Cytokine Release Syndrome Management Guidance

In the event symptoms of cytokine release syndrome occur, (fever, chills, nausea, vomiting and hypotension), despite pre-treatment, consider non-steroidal anti-inflammatory drugs (NSAIDs), repeat acetaminophen treatment, and repeat anti-pyretic or histamine receptor antagonist treatment. IVF boluses should also be considered to support symptomatic hypotension.

For symptoms of cytokine release syndrome (including hypotension and hypoxia) that persists and is unresponsive to above treatment, corticosteroid treatment with short biological half-life as per guidance in Table 11 should be considered. Additionally, vasopressor support and/or tocilizumab per institutional guidelines should be considered.

6.1.5 Excluded Treatments, Medical Devices, and/or Procedures During Study Period

The following treatments and/or procedures are excluded during the treatment period of the study:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in the protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than NL-201
- Radiation therapy with the following exceptions:
  - Radiation therapy to a symptomatic, non-target lesion or to the brain may be considered after consultation with the Sponsor and medical monitor approval
  - The patient must have clear measurable disease outside of the radiated field
- Live or live-attenuated vaccines (Note: Killed vaccines are allowed.)
- Systemic immunosuppressive therapy, with the following exceptions will be allowed:
  - Stable replacement doses of prednisone ≤ 10 mg/day [or equivalent]
  - Stress dosing treatment of patients with adrenal insufficiency
  - Study drug pre-medications for prophylaxis of study drug-related toxicities

- For treatment of any AE (see Table 11 for guidance), provided that the steroid dose is tapered to ≤ 10 mg/day prednisone (or equivalent) prior to receiving the next dose of NL-201

- Any other investigational agent, vaccine, or device
- Any major surgery, unless approved by Medical Monitor

If the Investigator determines that a participant requires any of the aforementioned treatments for any reason, study intervention (NL-201 and for Parts 3 and 4, pembrolizumab) must be discontinued.

6.2 Dose Modification

6.2.1 Dose-Cohort Study Escalation, De-Escalation, and Stopping Rules

6.2.1.1 Part 1: Dose Escalation Study of NL-201 Monotherapy

In Part 1, an mTPI[25] will be used to guide dose escalation and de-escalation within each schedule (ie, IV Schedule A, IV Schedule B), with the final decision regarding dose assignment being made by the DMC. In IV Schedule A, patients will be dosed every 21 days, while in IV Schedule B patients will be dosed on Days 1 and 8 of every 21-day cycle. The mTPI design matrix is shown in Figure 5.

Figure 5 mTPI Design Matrix for Part 1

	Number of enrollment at current dose level													
Number of DLT	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	E	E	E	E	E	E	E	E	E	E	E	E	E	E
1	S	S	S	S	E	E	E	E	E	E	E	E	E	E
2	DU	D	S	S	S	S	S	S	E	E	E	E	E	E
3		DU	DU	D	S	S	S	S	S	S	S	S	S	E
4			DU	DU	DU	D	S	S	S	S	S	S	S	S
5				DU	DU	DU	DU	D	S	S	S	S	S	S
6					DU	DU	DU	DU	DU	D	S	S	S	S
7						DU	DU	DU	DU	DU	DU	D	S	S
8							DU	DU	DU	DU	DU	DU	DU	DU
9								DU	DU	DU	DU	DU	DU	DU
10									DU	DU	DU	DU	DU	DU
11										DU	DU	DU	DU	DU
12											DU	DU	DU	DU
13												DU	DU	DU
14													DU	DU
15														DU

D = De-escalate to the next lower dose; DLT = dose-limiting toxicity; DU = current dose is unacceptably toxic; E = Escalate to the next higher dose; mTPI = modified toxicity probability interval; S = stay at the current dose
Target toxicity rate = 33%
Flat noninformative prior Beta (1,1) is used as a prior and $\varepsilon_1 = \varepsilon_2 = 0.05$

Assessment of toxicity and dose escalations or de-escalation as guided per the mTPI will be made on an ongoing basis based upon DLT-evaluable patients as defined by occurrence of DLT or completion of 21 days on study, and after review by the DMC. If dose escalation is indicated per mTPI, the DMC will recommend escalation to the next planned dose or additional dose based on review of observed toxicity. Dose escalation and de-escalation will occur independently foreach schedule. Intrasubject dose escalation to the next highest dose will be permitted once that dose level is cleared by the DMC in cases where doses have been tolerated by the patient and with Medical Monitor approval (see Table 8 for a proposed dose escalation scheme).

Table 8 Sample Dose Escalation Scheme for Part 1 IV

Monotherapy Cohorts (21-day cycles)		NL-201 dose level (µg/kg)	Maximum dose that may be administered at 1 time (µg)[a]
Schedule A (D1)	Schedule B (D1+D8)		
Planned NL-201 Dose Levels[b]			
1A	1B	0.1	10
2A	2B	0.3	30
3A	3B	1.0	100
4A	4B	3.0	300
5A	5B	6.0	600
6A	6B	12.0	1200
Potential NL-201 Intermediate Dose Levels[b]			
2A-1	2B-1	0.6	60
3A-1	3B-1	1.5	150
3A-2	3B-2	2.0	200
4A-1	4B-1	4.0	400
4A-2	4B-2	5.0	500
5A-1	5B-1	7.5	750
5A-2	5B-2	9.0	900
5A-3	5B-3	10.0	1000

D = day; DMC = Data Monitoring Committee; IV = intravenous.
a Applicable to patients that weigh ≥ 100 kg (dose is capped as to not allow > 100 times the current dose level to be administered to a patient at 1 time).

b At the recommendation of the DMC or based on Sponsor's determination, additional dose levels may be tested and/or cohorts may be expanded.

The maximum tolerated dose (MTD) is defined as the highest dose for which the probability of a patient experiencing a DLT with NL-201 during Cycle 1 is ≤ 33%. At the discretion of the DMC, and in keeping with the pre-specified mTPI design matrix, additional dose levels may be tested to determine the MTD.

Based on review of DLTs, the DMC may initiate step-dosing to improve the tolerability of NL-201, wherein 1 or more step-doses below the target dose would be provided prior to reaching the target dose (see Section 6.2.1.4). If step-dosing is used, the DLT window would extend from the first dose to 21 days after receiving the target dose (ie, would include the initial reduced-dose cycles in addition to the first target-dose cycle). Once a patient receives the intended target dose for their cohort, subsequent doses for that patient will continue at that dose level unless a delay in dosing of > 6 weeks causes washout of the drug, in which case the step-dosing regimen would be repeated if the patient is to resume dosing.

Supported by the mTPI design, the DMC will determine the RP2D and treatment schedule (IV Schedule A, IV Schedule B) for Part 2 by considering the PK, PDn, tolerability, antitumor activity, and the occurrence of any cumulative toxicities over multiple cycles of NL-201.

6.2.1.2 Part 3: Dose Escalation Study of NL-201 in Combination with Pembrolizumab

In Part 3, an mTPI[25] will be used to guide dose escalation and de-escalation for NL-201 within each schedule, with the final decision regarding dose assignment being made by the DMC. In Schedule A, patients will be dosed every 21 days, while in Schedule B patients will be dosed on Days 1 and 8 of every 21day cycle. The mTPI design matrix is shown in Figure 6.

Figure 6 mTPI Design Matrix for Part 3

	Number of enrollment at current dose level													
Number of DLT	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	E	E	E	E	E	E	E	E	E	E	E	E	E	E
1	S	S	S	S	E	E	E	E	E	E	E	E	E	E
2	DU	D	S	S	S	S	S	S	S	E	E	E	E	E
3		DU	DU	D	S	S	S	S	S	S	S	S	S	S
4			DU	DU	DU	D	D	S	S	S	S	S	S	S
5				DU	DU	DU	DU	DU	D	S	S	S	S	S
6					DU	DU	DU	DU	DU	DU	D	S	S	S
7						DU	DU	DU	DU	DU	DU	DU	D	D
8							DU	DU	DU	DU	DU	DU	DU	DU
9								DU	DU	DU	DU	DU	DU	DU
10									DU	DU	DU	DU	DU	DU
11										DU	DU	DU	DU	DU
12											DU	DU	DU	DU
13												DU	DU	DU
14													DU	DU
15														DU

D = De-escalate to the next lower dose; DLT = dose limiting toxicity; DU = current dose is unacceptably toxic;
E = Escalate to the next higher dose; mTPI = modified toxicity probability interval; S = stay at the current dose
Target toxicity rate = 30%
Flat noninformative prior Beta (1,1) is used as a prior and $\varepsilon_1 = \varepsilon_2 = 0.03$

Assessment of toxicity and dose escalations or de-escalation as guided per the mTPI will be made on an ongoing basis based upon DLT-evaluable patients as defined by occurrence of DLT or completion of 21 days on study, and after review by the DMC. If dose escalation is indicated per mTPI, the DMC will recommend escalation to the next planned dose or to an intermediate dose based on review of observed toxicity. Dose escalation and de-escalation will occur independently for Schedules A and B, and a given dose level must be determined by the DMC to be safe on Schedule A prior to enrolling on Schedule B. Dose levels will be escalated more gradually at higher dose levels (see Table 9 for a proposed dose escalation scheme).

Table 9 Sample Dose Escalation Scheme for Part 3

Combination Cohorts (21-day cycles)			NL-201 dose level (µg/kg)	Maximum dose of NL-201 that may be administered at 1 time (µg)[a]
Schedule A (D1)	Schedule B (D1+D8)	Pembrolizumab (D1)		
Planned NL-201 Dose Level				
P-1A	P-1B	200 mg	0.3	30
P-2A	P-2B	200 mg	1	100
P-3A	P-3B	200 mg	3	300
P-4A	P-4B	200 mg	6	600
P-5A	P-5B	200 mg	12	1200
Potential NL-201 Intermediate Dose Level[b]				
P-2A-1	P-2B-1	200 mg	0.6	60
P-3A-1	P-3B-1	200 mg	1.5	150
P-3A-2	P-3B-2	200 mg	2.0	200
P-4A-1	P-4B-1	200 mg	4.0	400
P-4A-2	P-4B-2	200 mg	5.0	500
P-5A-1	P-5B-1	200 mg	7.5	750
P-5A-2	P-5B-2	200 mg	9.0	900
P-5A-3	P-5B-3	200 mg	10.0	1000

D = day; DMC = Data Monitoring Committee
a Applicable to patients that weigh ≥ 100 kg (dose is capped as to not allow > 100 times the current dose level to be administered to a patient at 1 time)
b At the recommendation of the DMC or based on Sponsor's determination, additional dose levels may be tested and/or cohorts may be expanded.

The MTD is defined as the highest dose for which the probability of a patient experiencing a DLT with NL-201 during Cycle 1 is ≤ 33%. At the discretion of the DMC, and in keeping with the pre-specified mTPI design matrix, additional dose levels may be tested to determine the RP2D (see Table 9).

Based on review of DLTs, the DMC may initiate step-dosing to improve the tolerability of NL-201, wherein 1 or more step-doses below the target dose would be provided prior to reaching the target dose (see Section 6.2.1.4). If step-dosing is used, the DLT window would extend from the first dose to 21 days after receiving the target dose (ie, would include the initial reduced-dose cycles in addition to the first target-dose cycle). Once a patient receives the intended target dose for their cohort, subsequent doses for that patient will continue at that dose level unless a delay in dosing of > 6 weeks causes washout of the drug, in which case the step-dosing regimen would be repeated if the patient is to resume dosing.

Supported by the mTPI design, the DMC will determine the RP2D for NL-201 in combination with pembrolizumab and treatment schedule (either A or B) for Part 4 by considering the PK, PDn, and any cumulative toxicities over multiple cycles of NL-201 in combination with pembrolizumab.

6.2.1.3 Dose-Limiting Toxicity

Part 1

DLT must occur within Cycle 1 (< 21 days from first dose), or prior to completion of 21 days after first administration of NL-201 or after reaching the cohort target dose (CTD) in patients receiving step-dosing (see Appendix 1 [Section 10.1]). DLT is defined by the following:

- Any death not clearly due to the underlying disease or extraneous causes
- Hy's law:
  - AST/ALT > 3 × ULN, and
  - TBL > 2 × ULN, and
  - Alkaline phosphatase < 2 × ULN without alternative etiology
  - No other reason can be found to explain the combination of increased ALT/AST and TBL, such as viral hepatitis A, B, or C; preexisting or acute liver disease; or another drug capable of causing the observed hepatocellular injury
- Neutropenic fever
- ≥ Grade 4 neutropenia or thrombocytopenia > 5 days
- ≥ Grade 3 thrombocytopenia with bleeding
- ≥ Grade 4 vomiting/diarrhea of any duration
- ≥ Grade 3 autoimmune disorder. Examples include, but are not limited to, treatment-emergent myasthenia gravis, toxic thyroiditis, bullous pemphigoid, and Stevens-Johnson Syndrome
- ≥ Grade 3 fatigue ≥ 1 week
- ≥ Grade 3 electrolyte abnormalities that lasts > 72 hours or are symptomatic
- ≥ Grade 3 lipase and amylase if associated with symptoms of pancreatitis
- ≥ Grade 3 AST or ALT elevation lasting ≥ 14 days
- Any other ≥ Grade 3 non-hematologic toxicity that is at least possibly related to the study drug

- Recurrent Grade 3 infusion-related reaction despite pre-medication, or Grade 4 infusion-related reaction on the first occurrence
- Dose delay of > 14 days for the study drug due to an AE

All AEs of the specified grades will be considered DLTs except those that are clearly and incontrovertibly due to disease progression or extraneous causes.

Part 3

All toxicities will be graded using Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 based on the investigator assessment. The DLT window of observation will be during Cycle 1.

The occurrence of any of the following toxicities during Cycle 1 will be considered a DLT, if assessed by the investigator to be possibly, probably, or definitely related to study treatment administration.

- Grade 4 nonhematologic toxicity (not laboratory).
- Grade 4 hematologic toxicity lasting ≥ 7 days, except thrombocytopenia:
    - Grade 4 thrombocytopenia of any duration
    - Grade 3 thrombocytopenia associated with clinically significant bleeding
- Any nonhematologic AE ≥ Grade 3 in severity should be considered a DLT, with the following exceptions: Grade 3 fatigue lasting ≤ 3 days; Grade 3 diarrhea, nausea, or vomiting without use of anti-emetics or anti-diarrheals per standard of care; Grade 3 rash without use of corticosteroids or anti-inflammatory agents per standard of care.
- Any Grade 3 or Grade 4 non-hematologic laboratory value if:
    - Clinically significant medical intervention is required to treat the participant or
    - The abnormality leads to hospitalization, or
    - The abnormality persists for > 1 week.
    - The abnormality results in a drug-induced liver injury (DILI)
    - Exceptions: Clinically nonsignificant, treatable, or reversible laboratory abnormalities including liver function tests, uric acid, etc.
- Febrile neutropenia Grade 3 or Grade 4:
    - Grade 3 is defined as ANC < 1000/mm^3 with a single temperature of > 38.3°C (101°F) or a sustained temperature of ≥ 38°C (100.4°F) for more than 1 hour
    - Grade 4 is defined as ANC < 1000/mm^3 with a single temperature of > 38.3°C (101°F) or a sustained temperature of ≥ 38°C (100.4°F) for more than 1 hour, with life-threatening consequences and urgent intervention indicated.
- Prolonged delay (> 2 weeks) in initiating Cycle 2 due to treatment-related toxicity.
- Any treatment-related toxicity that causes the participant to discontinue treatment during Cycle 1.
- Missing > 25% of NL-201 doses as a result of drug-related AE(s) during the first cycle.
- Grade 5 toxicity.

6.2.1.4 Step-Dosing

Pre-clinical studies of NL-201 suggest that symptomatic toxicity of NL-201 in animals peaks with the first dose, such that symptomatic toxicity caused by subsequent doses is attenuated compared to that caused by the first dose. Dose levels that are not tolerated when given as an initial dose become tolerated after giving 1 or more initial step-doses at a reduced (tolerated) dose level, and remain tolerated in subsequent doses. Thus, if a specific dose level is not well tolerated (ie, elicits DLTs in ≥ 33% of patients), it is possible that tolerability can be improved by providing 1 or more reduced doses. Once the target dose is reached, subsequent doses would be given at the expected dose level for that cohort.

If step-dosing is implemented, dosing during the first cycle of step-dosing will be administered on Day 1 and Day 8 for both Schedule A and Schedule B patients, though in subsequent cycles Schedule A patients will resume Q3W dosing while Schedule B patients will continue Day 1+8 dosing.

The Cycle 1 dose used for step-dosing will be calculated as one half of the highest tolerated initial dose (HTID), ie, the highest initial dose at which < 33% of patients experience DLTs. Subsequent cycles will increase 1 dose level (DL) at a time to the CTD.

Table 10 Step-Dosing Guidance

	Cycle 1				
Steps	**D1**	**D8**	**C2**	**C3**	**C4**
1	0.5 x HTID	0.5 x HTID	CTD		
2	0.5 x HTID	0.5 x HTID	HTID + 1 DL	CTD	
3	0.5 x HTID	0.5 x HTID	HTID + 1 DL	HTID + 2 DL	CTD

C = cycle; CTD = cohort target dose; D = day; DL = dose level; HTID = highest tolerated initial dose

The DLT window for each patient during step-dosing will extend to include the cycle in which the patient first receives the CTD. The DLT window for each cohort is highlighted in gray in Table 10.

6.2.1.5 Stopping Rules

Enrollment will be immediately suspended for any treatment-related Grade 5 toxicity. After review and discussion of all relevant data by the DMC, including benefit and risk, a proposal to amend or discontinue the study will be considered. No additional patients will be enrolled until the review has been completed, measures to improve patient safety have been implemented, and an amendment, if indicated, is approved.

6.2.2 Dosage Adjustments, Delays, Rules for Withholding or Restarting, Permanent Discontinuation

6.2.2.1 NL-201 Dosage Adjustments, Delays, Rules for Withholding or Restarting, Permanent Discontinuation

Patients that experience a DLT may be dose-reduced or withdrawn from the study after discussion with the medical monitor. The reason for dose change of NL-201 is to be recorded on each patient's case report form (CRF). Dose escalation patients and expansion patients may also experience delays, reductions and other dose adjustments. Dose may be delayed up to 21 days; any delay beyond 21 days must be approved by the medical monitor. If Cycle 2 is skipped, then PK, antitumor activity, cytokines, and PDn samples should still be collected at the next cycle according to the Cycle 2 schedule and documented as unscheduled visits.

Refer to Table 11 for guidance on toxicity management and dose modifications.

For Parts 3 and 4, refer to Section 6.2.2.2 for guidance on withholding NL-201 when given in combination with pembrolizumab.

Table 11 Dose Delay[a] and Modification Guidance for IV Administration

Potential Toxicity	Dose Modification if Grade ≤ 2	Dose Modification if Grade ≥ 3
Chills		Hold dose until Grade ≤ 2
Fever		Hold dose until Grade ≤ 2
Pruritis		No change
Nausea/vomiting/anorexia		Hold dose until Grade ≤ 2
Diarrhea		Hold dose until Grade ≤ 2
Gastritis/stomach upset		Hold dose until Grade ≤ 2
Malaise		Hold dose until Grade ≤ 2 if other Grade ≥ 3 toxicities occur simultaneously
Anemia		Hold dose until Grade ≤ 2 or return to baseline
Leukocytosis		Hold dose until leukocyte count < 100,000/mm^3
Thrombocytopenia		Hold dose until Grade ≤ 2
Edema/weight gain		No change
Hypotension		Hold dose until Grade ≤ 2, resume 1 dose level lower. Stop treatment if vasopressor support is required.
Capillary leak syndrome		Hold dose until Grade ≤ 2
Cytokine release syndrome	Hold dose until recovery to Grade 1. Pre-medication should be adjusted per Section 6.1.3. NL-201 dosing may be resumed at the same dose.	**Grade 3**: Hold dose until recovery to Grade 1. Pre-medication should be adjusted per Section 6.1.3. NL-201 dosing may be resumed at the same dose. If Grade 3 recurs and cannot be managed with supportive measures including the use of steroids with short biological half-life (eg, prednisone), dose must be reduced by one dose level for subsequent doses after discussion with Medical Monitor. **Grade 4**: Stop treatment
Dyspnea		Hold dose until Grade ≤ 2, resume 1 dose level lower. Stop treatment if ventilator support is required
Oliguria		Hold dose until Grade ≤ 2, resume 1 dose level lower
Increased creatinine		Hold dose until Grade ≤ 2, resume 1 dose level lower if peak is Grade 3, stop treatment if peak is Grade 4.
Renal failure		Stop therapy if requires dialysis
Pleural effusion		Hold dose until Grade ≤ 2
Bowel perforation		Stop treatment
Anxiety		Hold dose until Grade ≤ 2 or back to baseline, resume 1 dose level lower

Potential Toxicity	Dose Modification if Grade ≤ 2	Dose Modification if Grade ≥ 3
Confusion		Hold dose until Grade ≤ 2, resume 1 dose level lower
Agitation or combativeness		Hold dose until Grade ≤ 2, resume 1 dose level lower
Somnolence		Hold dose until Grade ≤ 2, resume 1 dose level lower
Insomnia		No change
Arrhythmia		Hold dose until Grade ≤ 2, resume 1 dose level lower. If not resolved back to baseline within 3 weeks, or normal sinus rhythm not achieved with chemical cardioversion, stop treatment.
Elevated troponin levels		Hold dose until Grade ≤ 2, resume 1 dose level lower. If changes in left ventricular function have not improved to baseline within 3 weeks, stop treatment.
Myocardial infarction		Stop treatment
Elevated transaminases[b]		Hold dose if Grade 4 without liver metastases. If changes have not improved to baseline within 3 weeks, stop treatment.
Hyperbilirubinemia		Hold dose if Grade 4 without liver metastases. If changes have not improved to baseline within 3 weeks, stop treatment.
Electrolyte imbalances		Hold dose until Grade ≤ 2
Neutropenia		Hold dose until Grade ≤ 2, resume 1 dose lower if ANC < 500 for ≥ 5 days
Febrile neutropenia		Stop treatment
Infusion-related reaction or hypersensitivity reaction	Stop infusion immediately. Consider treatment with diphenhydramine and/or acetaminophen. Infusion may be resumed after Grade 2 reaction has resolved with slower infusion rate. Pre-medication should be adjusted per Section 6.1.3.	**Grade 3**: Consider treatment with diphenhydramine and/or acetaminophen and/or systemic steroids per institutional standard practice. If infusion reaction occurs and it can be adequately managed with supportive measures, the patient may be continued on the study with pre-medication adjusted per Section 6.1.3 for subsequent doses. Otherwise, stop treatment. If Grade 3 recurs and cannot be managed with supportive measures including steroids, dose must be reduced by 1 dose level for subsequent doses. **Grade 4**: Stop treatment

ANC = absolute neutrophil count; IV = intravenous.

a Dose may be delayed up to 21 days; any delay longer than 21 days must be approved by medical monitor

b Refer to Section 10.5 if drug-induced liver injury is suspected.

6.2.2.2 Pembrolizumab Dosage Adjustments, Delays, Rules for Withholding or Restarting, Permanent Discontinuation

6.2.2.2.1 Dose Modification and Toxicity Management for Immune-Related AEs Associated with Pembrolizumab

AEs associated with pembrolizumab combination exposure, including coadministration with additional compounds may represent an immune-related response. These irAEs may occur shortly after the first dose or several months after the last dose of pembrolizumab combination treatment and may affect more than one body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of pembrolizumab combination treatment, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in Table 12.

Attribution of Toxicity:

When study interventions are administered in combination, attribution of an AE to a single component is likely to be difficult. Therefore, while the investigator may attribute a toxicity event to the combination, to NL-201 alone, or to pembrolizumab alone, for AEs listed in Table 12, both interventions must be held according to the criteria in Table 12.

Holding Study Interventions:

When study interventions are administered in combination, if the AE is considered immune-related, both interventions should be held according to recommended dose modifications.

Restarting Study Interventions:

Participants may not have any dose modifications (no change in dose or schedule) of pembrolizumab in this study, as described in Table 12.

If the toxicity does not resolve or the criteria for resuming treatment are not met, the participant must be discontinued from all study interventions.

If the toxicities do resolve and conditions are aligned with what is defined in Table 12, the combination of NL-201 and pembrolizumab may be restarted at the discretion of the investigator. In these cases where the toxicity is attributed to the combination or to NL-201 alone, re-initiation of pembrolizumab as a monotherapy may be considered after communication with and agreement by the Sponsor.

Table 12 Dose Modification and Toxicity Management Guidelines for Immune-Related Adverse Events Associated with Pembrolizumab

General instructions:

- Severe and life-threatening irAEs should be treated with IV corticosteroids followed by oral steroids. Other immunosuppressive treatment should begin if the irAEs are not controlled by corticosteroids.
- Pembrolizumab must be permanently discontinued if the irAE does not resolve or the corticosteroid dose is not ≤ 10 mg/day within 12 weeks of the last pembrolizumab-treatment.
- The corticosteroid taper should begin when the irAE is ≤ Grade 1 and continue at least 4 weeks.
- If pembrolizumab has been withheld, pembrolizumab may resume after the irAE decreased to ≤ Grade 1 after corticosteroid taper.

irAEs	Toxicity grade (CTCAE V5.0)	Action with pembrolizumab	Corticosteroid and/or other therapies	Monitoring and follow-up
Pneumonitis	Grade 2	Withhold	Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper Add prophylactic antibiotics for opportunistic infections	Monitor participants for signs and symptoms of pneumonitis Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Recurrent Grade 2, Grade 3 or 4	Permanently discontinue		
Diarrhea/Colitis	Grade 2 or 3	Withhold	Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus) Participants with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

AST or ALT elevation or Increased Bilirubin	Grade 2[a]	Withhold	Administer corticosteroids (initial dose of 0.5 to 1 mg/kg prednisone or equivalent) followed by taper	Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3[b] or 4[c]	Permanently discontinue	Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	New onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold[d]	Initiate insulin replacement therapy for participants with T1DM Administer antihyperglycemc in participants with hyperglycemia	Monitor participants for hyperglycemia or other signs and symptoms of diabetes
Hypophysitis	Grade 2	Withhold	Administer corticosteroids and initiate hormonal replacements as clinically indicated	Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue[d]		
Hyperthyroidism	Grade 2	Continue	Treat with nonselective beta-blockers (eg, propranolol) or thionamides as appropriate	Monitor for signs and symptoms of thyroid disorders
	Grade 3 or 4	Withhold or permanently discontinue[d]		
Hypothyroidism	Grade 2, 3 or 4	Continue	Initiate thyroid replacement hormones (eg, levothyroxine or liothyronine) per standard of care	Monitor for signs and symptoms of thyroid disorders
Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	Administer corticosteroids (prednisone 1 to 2 mg/kg or equivalent) followed by taper	Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Neurological Toxicities	Grade 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		

Myocarditis	Grade 2, 3 or 4	Permanently discontinue	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology and/or exclude other causes
Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology or exclude other causes
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		
All Other irAEs	Persistent Grade 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the event[e]		
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

AE(s) = adverse event(s); ALT = alanine aminotransferase; AST = aspartate aminotransferase; CTCAE = Common Terminology Criteria for Adverse Events; DRESS = drug reaction with eosinophilia and systemic symptoms; GI = gastrointestinal; ir = immune related; IV = intravenous; SJS = Stevens-Johnson Syndrome; T1DM = type 1 diabetes mellitus; TEN = Toxic Epidermal Necrolysis; ULN = upper limit of normal.

Note: Non-irAE will be managed as appropriate, following clinical practice recommendations.

a AST/ALT: >3.0 to 5.0 x ULN if baseline normal; >3.0 to 5.0 x baseline, if baseline abnormal; bilirubin:>1.5 to 3.0 x ULN if baseline normal; >1.5 to 3.0 x baseline if baseline abnormal

b AST/ALT: >5.0 to 20.0 x ULN, if baseline normal; >5.0 to 20.0 x baseline, if baseline abnormal; bilirubin:>3.0 to 10.0 x ULN if baseline normal; >3.0 to 10.0 x baseline if baseline abnormal

c AST/ALT: >20.0 x ULN, if baseline normal; >20.0 x baseline, if baseline abnormal; bilirubin: >10.0 x ULN if baseline normal; >10.0 x baseline if baseline abnormal

d The decision to withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. If control achieved or ≤ Grade 2, pembrolizumab may be resumed.

e Events that require discontinuation include but are not limited to: encephalitis and other clinically important irAEs (eg, vasculitis and sclerosing cholangitis).

6.2.2.2.2 Dose Modification and Toxicity Management of Infusion-Reactions Related to Pembrolizumab

Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab associated infusion reaction are provided in Table 13.

Table 13 Pembrolizumab Infusion Reaction Dose Modification and Treatment Guidelines

CTCAE Grade	Treatment	Pre-medication at Subsequent Dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.	None

Grade 2
Requires therapy or infusion interruption but responds promptly to symptomatic treatment (eg, antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤ 24 hrs.
	• Stop Infusion.
	• Additional appropriate medical therapy may include but is not limited to:
	  ○ IV fluids
	  ○ Antihistamines
	  ○ NSAIDs
	  ○ Acetaminophen
	  ○ Narcotics
	• Increase monitoring of vital signs as medically indicated until the participant 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 (eg, from 100 mL/hr. to 50 mL/hr.). Otherwise dosing will be held until symptoms resolve and the participant should be pre-medicated for the next scheduled dose.
	• Participants who develop Grade 2 toxicity despite adequate pre-medication should be permanently discontinued from further study drug treatment
		Participant may be pre-medicated 1.5h (± 30 minutes) prior to infusion of study intervention with:
		Diphenhydramine 50 mg po (or equivalent dose of antihistamine).
		Acetaminophen 500-1000 mg po (or equivalent dose of analgesic).

CTCAE Grade	Treatment	Pre-medication at Subsequent Dosing
Grades 3 or 4 **Grade 3:** Prolonged (ie, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (eg, renal impairment, pulmonary infiltrates) **Grade 4:** Life-threatening; pressor or ventilator support indicated	• Stop Infusion. • Additional appropriate medical therapy may include but is not limited to: o Epinephrine** o IV fluids o Antihistamines o NSAIDs o Acetaminophen o Narcotics o Oxygen o Pressors o Corticosteroids • Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. • Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. Participant is permanently discontinued from further study drug treatment.	No subsequent dosing

IV = intravenous; CTCAE = Common Terminology Criteria for Adverse Events; NSAID = non-steroidal anti-inflammatory drug
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the CTCAE V5.0 at http://ctep.cancer.gov.

6.2.2.2.3 Other Allowed Dose Interruption for Pembrolizumab

Pembrolizumab may be interrupted for situations other than treatment-related AEs such as medical or surgical events and/or unforeseen circumstances not related to study intervention. However, study intervention is to be restarted within 3 weeks or 21 days of the originally scheduled dose and within 42 days of the previously administered dose, unless otherwise discussed with the Sponsor. The reason for interruption is to be documented in the patient's study record.

6.3 Measures to Minimize Bias: Randomization and Blinding

6.3.1 Randomization

This is an open-label, dose escalation/dose expansion study; patients will not be randomized.

6.3.2 Blinding

This is an open-label study.

6.4 Study Drug Compliance

When patients are dosed at the site, they will receive study drug directly from the Investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents and recorded in the CRF. The dose of study drug and study patient identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study drug.

6.5 Concomitant Therapy

Necessary supportive care, including antibiotics, blood products, IV fluids, anti-diarrheals, anti-emetics will be allowed per local standard of care. All treatments that the investigator considers necessary for a participant's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care.

Any medication or vaccine (including over-the-counter or prescription medicines, and/or herbal supplements) that the patient is receiving at the time of enrollment or receives during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates (if known)
- Concomitant therapies of interest only and if available during the course of the study: dosage information including dose and frequency

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

All concomitant medications received within 28 days prior to the first dose of study treatment and up to 30 days after the last dose of study treatment will be recorded. All concomitant medications administered during SAEs or ECIs are to be recorded. SAEs and events of clinical interest (ECIs) are defined in Section 8.4.

Participants should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are outlined along with the dose modification guidelines in Section 6.2.2.2.1.

Note: If after the evaluation of the event, it is determined not to be related to pembrolizumab, the investigator does not need to follow the treatment guidance. Refer to Table 12 in Section 6.2.2.2.1 for guidelines regarding dose modification and supportive care.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

6.6 Intervention After the End of the Study

Neoleukin reserves the unilateral right, at its sole discretion, to determine whether to supply NL-201 and by what mechanism, after termination of the study and before the product(s) is/are available commercially.

Parts 3 and 4 only: Participants who are still on study intervention at the time of study completion/termination may continue to receive study intervention if they are experiencing clinical benefit. The continued access to study intervention will end when a criterion for discontinuation is met or 35 doses of pembrolizumab have been administered.

7 DISCONTINUATION OF STUDY TREATMENT AND PATIENT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Treatment

Patients can decline to continue receiving investigational product and/or other protocol-required therapies or procedures at any time during the study but continue participation in the study. If this occurs, the Investigator is to discuss with the patient the appropriate processes for discontinuation from investigational product or other protocol-required therapies and must discuss with the patient the possibilities for continuation of the Schedule of Events (see Section 1.3) including different options of follow-up (eg, in person, by phone/mail, through family/friends, in correspondence/communication with other treating physicians, from the review of medical records) and collection of data, including endpoints and AEs, as applicable and must document this decision in the patient's source documentation. Patients who have discontinued investigational product and/or other protocol-required therapies or procedures should not be automatically removed from the study. Whenever safe and feasible, it is imperative that patients remain on-study to ensure safety surveillance and/or collection of outcome data.

Reasons for removal from study drug may include any of the following:

- AEs (including any study intervention-related toxicity specified as a reason for permanent discontinuation as defined in the guidelines for dose modification due to AEs in Section 6.2.2)
- Pregnancy (see Section 8.3.5 and Section 10.4)
- Disease-state criteria (eg, progressive disease that requires discontinuation of the study drug)
- Protocol deviation
- Requires alternative therapy
- Sponsor decision
- Patient request to discontinue study drug

- Parts 3 and 4 only: Discontinuation of treatment may be considered for participants who have attained a confirmed CR and have been treated for at least 8 cycles (at least 24 weeks), receiving 2 cycles of the combination including 2 doses of pembrolizumab and at least 2 doses of NL-201 (if still being treated with combination therapy) beyond the date when the initial CR was declared.
- Parts 3 and 4 only: Completion of 35 administrations (approximately 2 years) with pembrolizumab

  Note: The number of administrations is calculated starting with the first dose of pembrolizumab.

See the Schedule of Events (Section 1.3) for data to be collected at the end of treatment visit and follow-up and for any further evaluations that need to be completed.

7.2 Discontinuation/Withdrawal From the Study

An Investigator may discontinue or withdraw a patient from the study for the following reasons:

- Sponsor decision
- Withdrawal of consent from study
- Death
- Lost to follow-up
- Other

At the time of discontinuing from the study, if possible, an end of treatment visit should be conducted, as shown in the Schedule of Events (Section 1.3). See Schedule of Events for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

The patient will be permanently discontinued both from the study drug and from the study at that time.

If the patient withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.

If a patient withdraws from the study, he/she may request destruction of any samples taken and not tested, and the Investigator must document this in the site study records. Any samples untested at withdrawal will be retained and tested if the patient does not request destruction of samples.

7.3 Lost to Follow-Up

A patient will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a patient fails to return to the clinic for a required study visit:

- The site must attempt to contact the patient and reschedule the missed visit as soon as possible and counsel the patient on the importance of maintaining the assigned visit schedule and ascertain whether or not the patient wishes to and/or should continue in the study.
- Before a patient is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the patient (where possible, 3 telephone calls and, if necessary, a certified letter to the patient's last known mailing address or local equivalent methods). These contact attempts should be documented in the patient's source documentation.
- Should the patient continue to be unreachable, he/she will be considered to have withdrawn from the study.
- For patients who are lost to follow-up, the Investigator can search publicly available records (where permitted) to ascertain survival status. This ensures that the data set(s) produced as an outcome of the study is/are as comprehensive as possible.

Discontinuation of specific sites or of the study as a whole are handled in Section 10.1.10.

8 STUDY ASSESSMENTS AND PROCEDURES

- Only patients who meet all inclusion and exclusion criteria specified in Section 5 will be enrolled in this study.
- Study procedures and their timing are summarized in the Schedule of Events (Section 1.3).
- Immediate safety concerns should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the patient should continue or discontinue study drug.
- Adherence to the study design requirements, including those specified in the Schedule of Events, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential patients meet all eligibility criteria. The Investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable. **ECOG and laboratory evaluations required for Cycle 1 Day 1 (within 48 hours prior to dosing) must conform to the following criteria prior to first dose of study drug on Cycle 1 Day 1:**

  1. Patients with ECOG performance status of 0 or 1
  2. Platelets $\geq 100{,}000/mm^3$
  3. ANC $> 1500/mm^3$
  4. Hemoglobin: Parts 1 and 2: ≥ 8.0 g/dL; Parts 3 and 4: ≥ 9.0 g/dL (NOTE: Criteria must be met without packed red blood cell transfusion within the prior 2 weeks. Participants can be on stable dose of erythropoietin [$\geq$ approximately 3 months]).
  5. Lymphocyte count $\geq 500/mm^3$
  6. Eosinophils $< 1000/mm^3$

7. Renal function: eGFR ≥ 50 mL/min/1.73 m^2 as determined by 2009 CKD-EPI equation,[29] or creatinine WNL

8. AST and ALT: Parts 1 and 2: ≤ 3x ULN, or ≤ 5x ULN if known liver malignancy or metastasis; Parts 3 and 4: ≤ 2.5x ULN, or ≤ 5x ULN if known liver malignancy or metastasis

9. TBL WNL, or ≤ 3x ULN if known Gilbert's syndrome

10. Coagulation: INR OR PT ≤ 1.5 x ULN

11. Highly sensitive pregnancy test (serum or urine) confirming no pregnancy

- Procedures conducted as part of the patient's routine clinical management (eg, blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes, provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the Schedule of Events.

8.1 General Study Periods

8.1.1 Screening and Enrollment

Informed consent must be obtained before completing any screening procedure, discontinuation of standard therapy for any disallowed therapy, or obtaining sensitive medical or social information (eg, HIV-positivity status). After the patient has signed the ICF, the study staff registers the patient in screening log or CRF in order to assess eligibility for participation. Patients who meet all inclusion criteria and none of the exclusion criteria are eligible for enrollment into this study. **All screening evaluations must be performed within 28 days prior to Cycle 1 Day 1.**

8.1.2 Treatment Period

Visits will occur per the Schedule of Events (Section 1.3). The date of the first dose of protocol-required therapies is defined as Day 1. All subsequent doses and study visits will be scheduled based on the Day 1 date. Administration of protocol-required therapies is to be administered last during each visit that it is required, unless otherwise indicated in the Schedule of Events.

After discontinuation from study treatment, patients in Parts 2 and 4, who do not have confirmed disease progression are required to continue disease response assessments and report new anti-cancer treatment per the Schedule of Events (Section 1.3) and Section 8.1.5. Frequency of these disease response assessments will continue per the Schedule of Events until first subsequent anti-cancer treatment, death, lost to follow-up, withdrawal of consent, or confirmed disease progression, whichever comes first. Following confirmed disease progression, all patients enter long-term follow-up and will be assessed as per the Schedule of Events (Section 1.3) and Section 8.1.5.

8.1.3 End of Treatment

Upon permanent discontinuation of study drug for any reason, an end of treatment visit should be scheduled as soon as possible, but within 30 days following cessation of study treatment or

before patient initiates new anti-cancer therapy, whichever is earlier. For Parts 1 and 3 (dose escalation), if study treatment is discontinued for reasons other than death, withdrawal of consent, lost to follow-up, or study closure, a CT scan should be performed at EOT visit (within + 2 weeks) if a post-baseline CT scan has not been performed within 5 weeks prior to the EOT visit. Adverse events and concomitant medication will be followed up to 30 days after last dose of NL-201.

For Parts 3 and 4, all AEs will be followed up to 30 days following cessation of study treatment and all SAEs will be followed up to 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anti-cancer therapy, whichever is earlier.

Patients who discontinue from treatment due to progressive diseased based on RECIST or iRECIST or initiation of new anti-cancer therapy will enter long-term follow-up and will be followed per the Schedule of Events (Section 1.3) and Section 8.1.5.

8.1.4 Radiological Follow-Up

For Parts 2 and 4 (expansion cohorts), patients who discontinue from treatment due to reasons other than progressive disease based on RECIST or iRECIST will be followed in radiologic follow-up for documented progressive disease by RECIST and will enter long-term follow-up. If subsequent anti-cancer therapies are initiated during the period of time between discontinuation of treatment and progressive disease documentation, the patient will no longer be radiologically assessed for progression and will enter long-term follow-up.

8.1.5 Long-Term Follow-Up

Once confirmed disease progression (by RECIST 1.1 or iRECIST) or initiation of new anti-cancer therapy beyond NL-201, patients enter long-term follow-up. The site will call patients every 6 months (± 1 month) to assess survival. Patients in Parts 2 and 4 will also have any subsequent anti-cancer therapies recorded during long-term follow-up. Long-term follow-up will continue until death, withdrawal of consent, lost to follow-up, or study closure, whichever occurs first, for a maximum of 2 years after end of treatment. The total study duration, including all follow-up, is expected to be approximately 5 years.

8.2 Efficacy Assessments

Planned time points for all efficacy assessments are provided in the Schedule of Events (Section 1.3).

8.2.1 Assessment of Clinical Outcome: Imaging

Clinical outcome is measured with tumor assessment/response related endpoints as assessed according to RECIST 1.1,[30] and additionally, as assessed according to RECIST for use in cancer immunotherapy trials (iRECIST).[31]

At each tumor assessment visit, all images (as scheduled by the study protocol and additionally, all images as medically indicated) will be interpreted (assessment of response) by a radiologist according to RECIST 1.1 and additionally according to iRECIST. In Part 1, assessments will be performed locally. In Part 2, images must be sent to the central lab for assessment.

It is at the discretion of the treating Investigator whether to withdraw the patient from the study based on disease progression as determined by tumor assessment according to RECIST 1.1 or iRECIST or, in exceptional cases, based on clinical signs of progression.

Imaging for tumor response assessment is performed as indicated in the Schedule of Events (Section 1.3). During screening, baseline imaging by computed tomography (CT) of at least the chest, abdomen, and pelvis must be performed within 28 days prior to Cycle 1 Day 1. Radiologic evaluation of the brain is required for patients with history of CNS metastasis, or neurologic abnormality that suggests possible CNS metastasis or malignancy. Depending on the tumor entity, and if clinically indicated, imaging of further areas (eg, bone) should be conducted.

During the treatment period (and long-term follow-up in Parts 2 and 4 if study treatment was discontinued for reasons other than disease progression), CT of at least chest, abdomen, pelvis, and all areas affected with tumor lesions according to baseline imaging is performed. If clinically indicated, imaging of the brain and further areas should be conducted. Radiological imaging can also be done at additional times, if clinically indicated. All radiological images should be considered for tumor response assessment. If the patient cannot tolerate CT-contrast agents, assessments may be performed using magnetic resonance imaging (MRI). In a given patient, the same imaging modality and technique (eg, slice thickness, field of view) should be used for all scans during the study (if unable to use the same imaging modality and technique, the corresponding data will be excluded from the efficacy analysis).

For Parts 2 and 4 radiologic follow-up should continue until radiologic disease progression, start of a new anti-cancer treatment, loss to follow-up, or death.

CR and PR require confirmation by a repeat assessment no earlier than 4 weeks after the imaging criteria for response is first met.

Tumor images to determine tumor assessment/response rates and PFS will be evaluated based on RECIST 1.1 and additionally on iRECIST. For more detailed guidance on RECIST 1.1 please refer to the original publication[30] and 2 further publications should be considered in case of need for further definitions.[32,33]

For details regarding methods of lesion measurement and tumor response, see Section 10.6.

8.3 Safety Assessments

Planned time points for all safety assessments are provided in the Schedule of Events (Section 1.3).

8.3.1 Physical Examinations

- Screening and end of treatment visits: A complete physical examination will include, at a minimum, assessments of the following body parts/systems: skin, abdomen, extremities, head, neck, heart, lungs, lymph nodes (if palpable) and neurological systems.
- All other time points: A brief physical examination will include, at a minimum, assessments of the skin, lungs, abdomen, and cardiovascular system.

8.3.2 Vital Signs

- Temperature, pulse rate, oxygen saturation, blood pressure, and respiration rate will be assessed.
- Vital signs will be measured in a semi-supine position after 5 minutes rest. Blood pressure will include both systolic and diastolic blood pressures.

8.3.3 Electrocardiograms

- 12-lead ECG will be obtained as outlined in the Schedule of Events (see Section 1.3) using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals.

8.3.4 Clinical Safety Laboratory Assessments

- See Section 10.2 for the list of clinical laboratory tests to be performed and to the Schedule of Events (Section 1.3) for the timing and frequency.
- The Investigator must review the laboratory report, document this review, and record in the AE section of the CRF any clinically relevant changes occurring during the study. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those that are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the patient's condition.
- All laboratory tests with values considered clinically significantly abnormal during participation in the study (Cycle 1 Day 1 through 30 days after the last dose of NL-201) should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or medical monitor.
    - If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified and the Sponsor notified.
    - All protocol-required laboratory assessments, as defined in Section 10.2, must be conducted in accordance with the laboratory manual and the Schedule of Events.
    - If laboratory values from non-protocol specified laboratory assessments performed at the institution’s local laboratory require a change in patient management or are considered clinically significant by the Investigator (eg, SAE, or AE, or dose modification), then the results must be recorded.

8.3.5 Pregnancy Testing

- Refer to Section 5.1 Inclusion Criteria for pregnancy testing entry criteria.
- Highly sensitive pregnancy testing should be conducted before dosing on Day 1 of every cycle and other time points per the Schedule of Events.
- Additional serum or urine pregnancy tests may be performed, as determined necessary by the Investigator or required by local regulation, to establish the absence of pregnancy at any time during the patient's participation in the study.

8.3.6 Vital Status

Vital status must be obtained for all patients within the limits of local law. This includes patients who may have discontinued study visits with or without withdrawing consent and should include interrogation of public databases, if necessary. If deceased, the date and reported cause of death should be obtained.

8.4 Adverse Events and Serious Adverse Events

The definitions of an AE and SAE can be found in Section 10.3.

Adverse event(s) will be reported by the patient (or, when appropriate, by a caregiver, or surrogate).

The Investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study drug or study procedures, or that caused the patient to discontinue the study drug or study (see Section 7).

8.4.1 Time Period and Frequency for Collecting Adverse Events and Serious Adverse Events Information

Adverse events related to study-required procedures will be collected from signing of the ICF until the first dose of study drug. All AEs will be collected during the treatment period and followed for 30 days after the final dose of NL-201. In Parts 3 and 4, SAEs will be collected for 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anti-cancer therapy, whichever is earlier.

Medical occurrences not related to study-required procedures that begin before the start of study drug but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the CRF not the AE section.

All SAEs will be recorded and reported to the Sponsor or designee immediately and under no circumstance should this exceed 24 hours, as indicated in Section 10.3. The Investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available.

Investigators are not obligated to actively seek AEs or SAEs after conclusion of the study participation. However, if the Investigator learns of any SAE, including a death, at any time after a patient has been discharged from the study, and he/she considers the event to be reasonably related to the study drug or study participation, the Investigator must promptly notify the Sponsor.

In general, an abnormal laboratory value should not be recorded as an AE unless it is associated with clinical sign or symptoms, requires intervention, results in a SAE, or results in study termination or interruption, delay or discontinuation of study treatment.

8.4.2 Events of Clinical Interest (ECI)

Selected non-serious and SAEs are also known as ECI and must be reported to the Sponsor within 24 hours of awareness.

Events of clinical interest for this trial include:

- An elevated AST or ALT lab value that is greater than or equal to 3 x the ULN and an elevated TBL lab value that is greater than or equal to 2 x the ULN and, at the same time, an alkaline phosphatase lab value that is less than 2 x the ULN, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.

  Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology. The trial site guidance for assessment and follow up of these criteria can be made available. It may also be appropriate to conduct additional evaluation for an underlying etiology in the setting of abnormalities of liver blood tests including AST, ALT, bilirubin, and alkaline phosphatase that do not meet the criteria noted above. In these cases, the decision to proceed with additional evaluation will be made through consultation between the study investigators and the Sponsor Clinical Director. However, abnormalities of liver blood tests that do not meet the criteria noted above are not ECIs for this trial.

8.4.3 Method of Detecting Adverse Events and Serious Adverse Events

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Section 10.3.

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the patient is the preferred method to inquire about AE occurrences.

8.4.4 Follow-up of Adverse Events and Serious Adverse Events

After the initial AE/SAE report, the Investigator is required to proactively follow each patient at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the patient is lost to follow-up (as defined in Section 7.3). Further information on follow-up procedures is given in Section 10.3.

8.4.5 Regulatory Reporting Requirements for Serious Adverse Events

- All SAEs must be reported within 24 hours of site awareness by the Investigator to the Sponsor so that legal obligations and ethical responsibilities towards the safety of patients and the safety of a study drug under clinical investigation are met.
- The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study drug under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and Investigators.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions according to local regulatory requirements and Sponsor policy and forwarded to Investigators as necessary.
- An Investigator who receives an Investigator safety report describing an SAE or other specific safety information (eg, summary or listing of SAEs) from the Sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

8.4.6 Pregnancy

- Details of all pregnancies in female patients and female partners of male patients and exposure during breastfeeding will be collected after the start of study drug and until 60 days after the last dose of NL-201 and 120 days after the last dose of pembrolizumab, or 30 days following cessation of study treatment if the patient initiates new anti-cancer therapy must be reported by the investigator.
    - If a participant inadvertently becomes pregnant while on study treatment the participant will be immediately discontinued from study treatment.
- If a pregnancy is reported, the Investigator should inform the Sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in Section 10.4.
- Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

8.5 Reporting and Treatment of Overdose

Any overdose of the Sponsor's product, as defined below, should be reported to the Sponsor within 24 hours of awareness, regardless if associated with clinical symptoms or abnormal laboratory results.

For this study, any dose of NL-201 greater than 20% of target dose within a 24-hour time period will be considered an overdose.

In the event of an overdose, the Investigator should:

1. Contact the Medical Monitor immediately.

2. Consider standard supportive therapy including, if necessary, hospitalization for observation and monitoring.
3. Closely monitor the patient for any AE/SAE and laboratory abnormalities.
4. Obtain a serum sample for PK analysis if requested by the Medical Monitor (determined on a case-by-case basis).
5. Document the quantity of the excess dose as well as the duration of the overdose in the CRF.

Decisions regarding dose modifications will be made by the Investigator in consultation with the Medical Monitor based on the clinical evaluation of the patient.

Parts 3 and 4 only: For this study, an overdose of pembrolizumab will be defined as any dose of 1000 mg or greater.

No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, the participant should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

8.6 Pharmacokinetics

Serial serum samples for PK analysis of NL-201 will be collected during Cycles 1 and 2 for IV Schedule A, IV Schedule B. In addition, trough samples (ie, pre-dose) and peak samples (ie, 1 hour after dosing) will be collected at prespecified time points as described in the Schedule of Events (Section 1.3). The primary purpose of collecting these samples is to measure the serum concentrations of NL-201 to characterize the PK of NL-201 after single- and multiple-dose administration. This information will also further inform the building of PK models to describe NL-201 PK and such models will be used to predict NL-201 serum levels under different dosing scenarios to help select an appropriate dose/schedule of NL-201. The dates and exact times of NL-201 administration before collecting PK samples and the dates and exact times of post-dose PK sample collections will be recorded. PK characterization will assess both NL-201 and the free protein.

8.7 Pharmacodynamics/Biomarkers

8.7.1 Exploratory Assessment of NL-201-Dependent Effects in Blood

Blood samples will be collected as indicated in the Schedule of Events (Section 1.3) to assess the PDn effect of NL-201 during therapy. Planned assays include flow-cytometric analysis on PBMCs as well as serum measurements of inflammatory cytokines.

8.7.2 Exploratory Assessment of NL-201-Dependent Effects on the Tumor Microenvironment (Patients who Have Consented to Tumor Biopsy Collection Part 1, Part 2 and Part 4)

To understand the relationship between the biological characteristics of tumors before treatment and patient outcomes, tissue from pre-treatment (archival or freshly obtained specimens) and on-treatment tumor biopsies will be examined. Biopsies will be assessed for specific PD,

predictive, and prognostic biomarkers in the tumor. To characterize the malignancy and response to study treatment, biomarker assessments in tumor biospecimens may include measurements of NL-201 potential metabolites, PD-L1, as well as characterization of the TME, drug target(s), tumor subtyping, and/or gene expression. Assays may include immunohistochemistry (IHC) and next generation sequencing of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA).

Tumor biopsies should be performed as indicated in the Schedule of Events (Section 1.3). Biopsies should be collected by appropriately trained clinical site personnel (eg, an interventional radiologist for internal tumor biopsies; trained personnel such as a dermatologist for cutaneous tumor biopsies). It is strongly recommended that a pathologist be present during biopsies when feasible to ensure sufficient tumor content of the biopsy location, and to confirm that biopsy acquisition and processing techniques are optimal.[34]

Tumor biopsies will be collected for the assessment of NL-201-mediated effects on the tumor microenvironment (TME), including paired pre- and post-treatment biopsies. Biopsy tissues for exploratory analysis should be in FFPE blocks when possible, and secondarily as slides. Analyses will include, but will not necessarily be limited to, transcriptional profiling and multiplex immunohistochemistry and to assess treatment-related changes in immune cell populations, phenotypes and spatial organization within the TME.

8.7.3 PD-L1 Expression (Part 4 Only)

Tumor tissue (archival or fresh biopsy) will be collected at screening to assess PD-L1 expression.

8.7.4 Tumor Mutational Burden Determination (Part 4 Only)

Tumor tissue (archival or fresh biopsy, as available) will be collected at screening to assess tumor mutational burden (TMB).

8.8 Immunogenicity Assessments

Antibodies to NL-201 will be evaluated in blood samples collected from all patients according to the Schedule of Events. These samples will be tested by the Sponsor's designee.

Blood samples will be screened for antibodies binding to NL-201 and the titer of confirmed positive samples will be reported. Other analyses may be performed to verify the stability of antibodies to NL-201 and/or further characterize the immunogenicity of NL-201.

The detection and characterization of antibodies to NL-201 will be performed using a validated assay method under the supervision of the Sponsor. All samples collected for detection of antibodies to study drug will also be evaluated for NL-201 serum concentration to enable interpretation of the antibody data. Antibodies may be further characterized and/or evaluated for their ability to neutralize the activity of the study drug(s).

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypothesis

For NL-201 monotherapy (Parts 1 and 2), no statistical hypothesis will be tested during dose escalation in Part 1. In Part 2, the null hypothesis that the response rate to NL-201 is ≤ 2.5% in each cohort will be tested against the alternative hypothesis that the response rate is ≥ 15%.

For NL-201 in combination with pembrolizumab (Parts 3 and 4), no statistical hypothesis will be tested during dose escalation in Part 3 and dose expansion in Part 4.

9.2 Sample Size Determination

In Part 1, 2 NL-201 monotherapy dosing schedules (IV Schedule A, IV Schedule B) will be evaluated with the goal being to determine the MTD of each schedule. The MTD is defined as the highest dose for which the probability of a patient experiencing a DLT with NL-201 is ≤ 33%. Based on the totality of data arising in Part 1, the DMC will determine a RP2D and treatment schedule of NL-201 for Part 2 that will not exceed the MTD.

Dose escalation in Part 1 will be guided by an mTPI design with up to approximately 50 enrolled per schedule as depicted in Figure 5. This design and number of patients per schedule provides a high likelihood of correctly identifying the MTD with approximately one half of the patients (ie, approximately n = 15) being exposed to the MTD, very few being exposed to lower, less effective doses and relatively few being exposed to the MTD+1 dose.

In addition, there will be backfill cohorts (up to approximately 12 patients in total) , at certain DMC-cleared dose levels and schedules, to collect, pharmacokinetic, pharmacodynamic and response data in certain tumor types or to explore additional pre-medication regimens (eg. steroids). The decision to enroll patients into backfill cohorts and to define the patients to be enrolled will be made by the Sponsor.

Note: “Enrolled” means any patient that has met all of the study inclusion and exclusion criteria and is eligible to receive study drug.

In Part 2, an expansion cohort study will evaluate safety and estimate antitumor activity of NL-201 as a monotherapy at the RP2D in 2 cohorts of patients: (1) melanoma and (2) RCC. A Simon’s 2-stage design will be used for each cohort independently to test the null hypothesis that the true monotherapy response rate is ≤ 2.5% vs the alternative that the true response rate is ≥ 15%. In the first stage, 18 patients will be evaluated. If 0/18 objective responses are seen, recruitment to the cohort will be stopped for futility. Otherwise, a further 12 patients will be evaluated for a total of 30 patients. This design provides 80% power and a Type I error rate of 0.031 under the stated null and alternative hypotheses.

In Part 3, with the mTPI study design, the exact number of patients needed to complete the dose escalation is unknown because it depends on the number of cohorts required to reach MTD and the number of patients in each cohort. Dose escalation will be conducted in up to 42 adult

patients (21 patients in each schedule) with advanced solid tumors to determine the safety profile, RP2D and treatment schedule of NL-201 in combination with pembrolizumab.

No formal hypothesis test is planned for Part 4 (dose expansion). The sample size of 30 is considered appropriate and sufficient to confirm the combination RP2D. The sample size for each cohort was based on the consideration of precision of estimates of ORR. For a sample size of 30, below is the summary of expected precision of ORR estimates. For example, if there is 6 responders observed among 30 patients treated, the observed ORR will be 20%, with 95% confidence interval of (7.7%, 38.6%).

Table 14 Observed ORR and 95% Confidence Interval with a Sample Size of 30

Number of Responders	Observed ORR	95% Confidence Interval*
6	20%	(7.7%, 38.6%)
9	30%	(14.7%, 49.4%)
12	40%	(22.7%, 59.4%)

ORR = objective response rate
* Based on Clopper-Pearson method (1934)

9.3 Populations for Analyses

For purposes of analysis, the following populations are defined in Table 15.

Table 15 Populations for Analyses

Population	Description
Safety Evaluable Population	For Parts 1 and 2, all patients who receive any amount of study drug (NL-201) will be considered evaluable for safety regardless of the duration of NL-201 treatment. This population is defined for Part 1, and Part 2. For Parts 3 and 4, all patients who receive any amount of study drug (NL-201 or pembrolizumab) will be considered evaluable for safety regardless of the duration of treatment. This population is defined for Part 1 and Part 3, and Part 2 and Part 4, expansion cohorts. This population will be used to summarize safety parameters.
Efficacy Evaluable Population	For Parts 1 and 2, all patients with measurable disease by RECIST, who receive any amount of study drug (NL-201) or who discontinue study drug early due to disease progression and/or death will be considered evaluable for efficacy. For Parts 3 and 4, all patients with measurable disease by RECIST, who receive any amount of study drug (NL-201 or pembrolizumab) or who discontinue study drug early due to disease progression and/or death will be considered evaluable for efficacy. This population is defined for Part 1 and Part 3, and Part 2 and Part 4.
DLT-Evaluable Population	Patients enrolled into Part 1 or Part 3 who either complete the first 21 days of study or who experience a DLT.

DLT = dose limiting toxicity; RECIST = Response Evaluation Criteria in Solid Tumors

9.4 Statistical Analyses

9.4.1 General Considerations

Demographic data and disease-related characteristics will be summarized separately for Part 1, by schedule (eg, IV Schedule A, IV Schedule B), and Part 2, by expansion cohort. Descriptive statistics (count and percent, mean, median, standard deviation, minimum, maximum) will be used. All patient data, efficacy and safety data will be summarized. Data from Parts 3 and 4 (NL-201 in combination with pembrolizumab) will be summarized in a similar manner.

A full Statistical Analysis Plan will be prepared to describe in full the statistical methods and analyses to be applied to the data arising in this study.

9.4.2 Determination of MTD in Part 1 and Part 3

For each schedule (IV Schedule A, IV Schedule B) in Part 1 (NL-201 monotherapy), the determination of MTD will be the highest dose for which the probability of a patient experiencing a DLT with NL-201 during Cycle 1 is ≤ 33%. Refer to Section 9.2 for more details.

Similarly, for each schedule separately in Part 3 (NL-201 in combination with pembrolizumab), the determination of MTD will be the highest dose for which the probability of a patient experiencing a DLT with NL-201 during Cycle 1 is ≤ 33%. Refer to Section 9.2 for more details.

9.4.3 Assessment of Preliminary Efficacy in Part 2 and Part 4

For NL-201 monotherapy, while tumor response data based on both RECIST 1.1 and iRECIST will be collected from all patients in dose escalation and expansion, formal analysis of tumor response will only be performed for the expansion cohorts in Part 2 based on iRECIST. Results will be presented and summarized using an exact 95% binomial confidence interval.

Other response-related efficacy parameters including time to response, duration of response, and clinical benefit rate will be also summarized by Part 2 expansion cohort.

In expansion cohorts in Part 2, OS and progression free survival (defined as the time from study entry to the time of disease progression or death from any cause) will be displayed graphically using Kaplan-Meier plots. Median PFS and OS will be extracted from the Kaplan-Meier plots along with their associated 95% confidence intervals. PFS and OS rates at 3 monthly intervals will also be extracted. Patients who do not experience progression or death will be censored at their last clinic visit contact for the assessment of disease progression.

For NL-201 in combination with pembrolizumab, data from Part 4 dose expansion cohort will be summarized and analyzed in a similar manner.

9.4.4 Pharmacokinetics

The PK concentrations and summary parameters of NL-201 will be listed, summarized and displayed graphically by schedules in Part 1 and the 2 expansion cohorts in Part 2.

Individual and mean serum concentration data will be plotted over time. Descriptive statistics will be presented for serum PK parameters including (but not limited to) C_{max}, t_{max}, AUC_t, AUC_{inf}, $t_{1/2}$, CL, volume of distribution (Vd), etc.

PK data collected in this study are intended to additionally contribute to population PK modeling. These models may be used to predict NL-201 PK in different dosing scenarios to further support the selection of a RP2D of NL-201. The population PK analyses may include data collected in additional NL-201 clinical studies. The analysis plan for population PK modeling will be separately developed and the results of such modeling will not be presented in the clinical study report for this study but will be presented in a separate report.

The PK data from Parts 3 and 4 will be summarized similarly.

9.4.5 Exploratory Evaluations

The relationship between tumor response and markers of immune cell activation and exhaustion, and also inflammatory cytokine levels and/or other biomarkers of interest will be explored via logistic regression. Data from Part 1, by schedules, and the 2 expansion cohorts in Part 2 will be included.

Similar exploratory analyses may be performed for data from Parts 3 and 4, as appropriate.

9.4.6 Safety Data

Safety data will be summarized for the safety evaluable population for Part 1 by schedule (eg, IV Schedule A, IV Schedule B), and Part 2; a summary will also be made by dose/schedule combining safety data from all parts of the study as appropriate. These data will include AEs and laboratory parameters. Adverse event terms will be coded using the Medical Dictionary for Drug Regulatory Activities (MedDRA®, version 23.0 or later). Adverse events will be summarized by body system, preferred term, severity, and relationship to treatment, with procedure-related events from Screening summarized separately from treatment-emergent AE. Serious adverse events, deaths, and AEs leading to early discontinuation of study drug will be summarized. Laboratory parameters will be summarized by maximum National Cancer Institute CTCAE severity grade and also by change from study entry to scheduled time points using descriptive statistics. Laboratory parameter listings will include the normal ranges for each parameter. Each value will be classified as falling above, below, or within the normal range.

Safety data from Parts 3 and 4 will be summarized similarly, with further details to be provided in Statistical Analysis Plan.

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

10.1.1 Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines
- Applicable International Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines
- Applicable laws and regulations

The protocol, protocol amendments, informed consent form (ICF), Investigator's Brochure, and other relevant documents (eg, patient recruitment advertisements) must be submitted to an Institutional Review Board (IRB)/Independent Ethics Committee (IEC) by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study patients.

The Investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of serious adverse events (SAEs) or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulation (CFR), ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

10.1.2 Financial Disclosure

Investigators and sub-Investigators will provide the Sponsor with sufficient, accurate financial information, as requested, to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

10.1.3 Informed Consent Process

- An initial sample ICF is provided for the Investigator to prepare the informed consent document to be used at his or her site. Updates to the sample ICF are to be communicated formally in writing from the Neoleukin Study Manager to the Investigator. The written ICF is to be prepared in the language(s) of the potential patient population. The ICF to be used at each site, and any changes to a site's ICF, must be approved by the Sponsor before use.
- The Investigator or his/her representative is responsible for presenting the nature of the study to the patient in lay language, as well as risks and alternate therapies, and answer all questions regarding the study, using the IRB/IEC approved informed consent document.
- Patients must be informed that their participation is voluntary. Patients will be required to sign a statement of informed consent that meets the requirements of Food and Drug Administration (FDA) CFR (21 CFR 50, 56, 312), Declaration of Helsinki (Brazil, 2015), local regulations, ICH guidelines, Health Insurance Portability and Accountability Act requirements, where applicable, and the IRB/IEC or study center.
- Source documentation must include a statement that written informed consent was obtained before the patient was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- If informed consent is obtained from a legally authorized representative for a patient who is unable to provide informed consent at study entry (if applicable), but the patient is later able to provide informed consent, the Investigator or his/her representative must obtain written informed consent from the patient.
- Patients must be re-consented to the most current version of the ICF(s) during their participation in the study.
- The original signed ICF must be retained in accordance with institutional policy, and a copy of the ICF(s) must be provided to the patient.

A patient who is rescreened is not required to sign another ICF if the rescreening occurs within 28 days from the previous ICF signature date.

10.1.4 Data Protection

- Patients will be assigned a unique identifier by the Sponsor. Any patient records or datasets that are transferred to the Sponsor will contain the identifier only; patient names or any information that would make the patient identifiable will not be transferred.
- The patient must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the patient who will be required to give consent for their data to be used as described in the informed consent.
- The patient must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.
- The Investigator must ensure that the patient's confidentiality is maintained for documents submitted to Neoleukin.

- For SAEs reported to Neoleukin, patients are to be identified by their unique patient identification number, initials (for faxed reports, in accordance with local laws and regulations), and age (in accordance with local laws and regulations).
- In compliance with governmental regulations/ICH GCP Guidelines, it is required that the Investigator and institution permit authorized representatives of the company, of the regulatory agency(s), and the IRB/IEC direct access to review the patient's original medical records for verification of study related procedures and data. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study.

10.1.5 Confidentiality

All information provided by the Sponsor and all data and information generated by the site as part of the study (other than a patient's medical records) will be kept confidential by the Investigator and other site staff. This information and data will not be used by the Investigator or other site personnel for any purpose other than conducting the study. These restrictions do not apply to: (1) information that becomes publicly available through no fault of the Investigator or site staff; (2) information that it is necessary to disclose in confidence to an IRB/IEC or other personnel as provided in this protocol; or (3) information that it is necessary to disclose in order to provide appropriate medical care to a study patient. If a written contract with a site for the conduct of the study that includes confidentiality provisions inconsistent with this statement is executed, that contract's confidentiality provisions shall apply for that site rather than this statement.

10.1.6 Retention of Source Data and Samples

Study data for all parts of this study must be retained until ≥ 25 years or per local agency requirements after the last marketing approval in an ICH region or ≥ 25 years or as per local agency requirements have elapsed since the formal discontinuation of clinical development of the investigational product (eg, via notification of the FDA or local regulatory authority). The Investigator/institution may not destroy records without written authorization from the Sponsor. If an Investigator leaves the institution at which the study was conducted, arrangements must be made to ensure another responsible party is designated to maintain the records.

Normally, these records will be held in the Investigator's archives. If the Investigator is unable to meet this obligation, he or she must ask the Sponsor for permission to make alternative arrangements. Details of these arrangements must be documented in writing to the Sponsor.

10.1.7 Dissemination of Clinical Study Data

The Sponsor will register and/or disclose the existence of and the results of clinical studies as required by local laws and regulations.

10.1.8 Data Quality Assurance

- All patient data relating to the study will be recorded on printed or electronic case report forms (CRFs) unless transmitted to the Sponsor or designee electronically (eg, laboratory

data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.

- The Investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections, and provide direct access to source data documents.
- Monitoring details describing strategy (eg, risk-based initiatives in operations and quality, such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities, and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.
- The Sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The Sponsor assumes accountability for actions delegated to other individuals (eg, Contract Research Organizations).
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of patients are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- CRFs must be completed in English. TRADENAMES® (if used) for concomitant medications may be entered in the local language. Consult the country-specific language requirements.

10.1.9 Source Documents

- Source documents provide evidence for the existence of the patient and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.
- Data reported on the CRF or entered in the electronic CRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.

10.1.10 Study and Site Start and Closure

The study start date is the date on which the clinical study will be open for recruitment of patients.

The first act of recruitment is the first site activated and will be the study start date.

The Sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The Investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines
- Inadequate recruitment of patients by the Investigator
- Discontinuation of further study drug development

If the study is prematurely terminated or suspended, the Sponsor will promptly inform the Investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator will promptly inform the patient and must ensure appropriate patient therapy and/or follow-up.

10.1.11 Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.
- The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.
- If a written contract with a site for the conduct of the study that includes publication provisions inconsistent with this statement is executed, that contract’s publication provisions shall apply for that site rather than this statement.

10.2 Appendix 2: Clinical Laboratory Tests

The tests detailed in Table 16 will be performed by the central laboratory and/or the local laboratory.

Protocol-specific requirements for inclusion or exclusion of patients are detailed in Section 5 of the protocol.

Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

Table 16 Protocol-Required Laboratory Assessments

Local Laboratory: Hematology	Local Laboratory: Clinical Chemistry	Local Laboratory: Urinalysis
Hematocrit Hemoglobin Hemoglobin A1c (screening only) Platelet count RBC indices: MCV MCH % Reticulocytes RBC count WBC count with differential: neutrophils lymphocytes monocytes eosinophils basophils	Albumin ALT/SGPT Alkaline phosphatase (ALP) AST/SGOT BUN Calcium Creatinine Glucose Potassium Sodium Total bilirubin Total protein	Microscopic examination (if blood or protein is abnormal) pH, glucose, protein, blood, ketones, by dipstick Specific gravity
		Local Laboratory: Coagulation
		INR PT aPTT
Local Laboratory: Viral Serology	**Local Laboratory: Other**	**Central Laboratory**
HIV antibody	Estradiol FSH Serum/urine pregnancy tests Parts 3 and 4: thyroid function tests: Triiodothyronine (T3) or Free Triiodothyronine (FT3) Free thyroxine (FT4) Thyroid stimulating hormone (TSH)	ADA Immune cell phenotyping Tumor tissue biopsy Cytokines PK PD-L1

ADA = antidrug antibody; ALT = alanine aminotransferase; aPTT = partial thromboplastin time; AST = aspartate aminotransferase; BUN = blood urea nitrogen; FSH = follicle-stimulating hormone; HIV = human immunodeficiency virus; INR = international normalized ratio; PK = pharmacokinetics; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; PD-L1 = programmed cell death ligand 1; PT = prothrombin time; RBC = red blood cell; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic-pyruvic transaminase; WBC = white blood cell

Investigators must document their review of each laboratory safety report.

10.3 Appendix 3: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-Up, and Reporting

10.3.1 Definition of Adverse Event

Adverse Event Definition:

- An adverse event (AE) is any untoward medical occurrence in a clinical study patient, temporally associated with the use of the study drug, whether or not considered related to the study drug.
    - When recording an AE resulting from a laboratory abnormality, the resulting medical condition rather than the abnormality itself should be recorded (eg, record "anemia" rather than "low hemoglobin").

  NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of the study drug.

Events <u>Meeting</u> the Adverse Event Definition:

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, electrocardiogram [ECG], radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator (ie, not related to progression of underlying disease).
- Exacerbation of a chronic or intermittent preexisting condition including either an increase in frequency and/or intensity of the condition.
- New condition detected or diagnosed after study drug administration even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as an AE or SAE if they fulfil the definition of an AE or SAE.

Events Not Meeting the Adverse Event Definition:

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments that are associated with the underlying disease or preexisting condition, unless judged by the Investigator to be more severe than expected.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the patient's condition.
- Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of preexisting disease(s) or condition(s) present or detected at the start of the study that do not worsen.

10.3.2 Definition of Serious Adverse Event

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

Serious Adverse Event Definition:

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

a. Results in death

b. Immediately life-threatening

   The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at immediate risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization

   In general, hospitalization signifies that the patient has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

   Hospitalization for elective treatment of a preexisting condition that did not worsen from baseline is not considered an AE.

d. Results in refractory disability/incapacity

   The term disability means a substantial disruption of a person's ability to conduct normal life functions.

   This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) that may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other medically significant serious event:

Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require medical or surgical intervention to prevent 1 of the other outcomes listed in the above definition. These events should usually be considered serious.

Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.3.3 Recording and Follow-Up of Adverse Events and/or Serious Adverse Events

Adverse Event and Serious Adverse Event Recording:

- When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The Investigator will then record all relevant AE/SAE information in the AE/SAE Report Form and supporting case report forms (CRFs) (eg, Medical History, and Concomitant Medications).
- It is **not** acceptable for the Investigator to send photocopies of the patient's medical records to the Sponsor or designee in lieu of completion of the AE/SAE Report Form.
- There may be instances when copies of medical records for certain cases are requested by the Sponsor or designee. In this case, all patient identifiers, with the exception of the patient number, will be redacted on the copies of the medical records before submission.
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
- The Investigator must assign the following AE attributes:
  - AE diagnosis or syndrome(s), if known (if not known, signs or symptoms)
  - Dates of onset and resolution (if resolved)
  - Intensity (or toxicity defined below)
  - Assessment of relatedness to study drug or other protocol-required therapies
  - Action taken
- If AE severity changes, record each change as a single event.
- The following should be considered when recording SAEs:
  - Death is an outcome of an event. The event that results in the death should be recorded and reported on both the SAE Report Form and CRF. Deaths due to disease progression will not be classified as an AE and should be recorded in the CRF on the Death Form.
  - For hospitalizations, surgical, or diagnostic procedures, the illness leading to the surgical or diagnostic procedure should be recorded as the SAE, not the procedure itself.

- There are special instructions for reporting of cytokine release syndrome. For any events of cytokine release syndrome, instead of reporting a general term of cytokine release syndrome, please report all signs and symptoms of cytokine release syndrome (eg, fever, chills/rigors, vomiting, tachycardia, headache, rash, arthralgia, myalgia, chest discomfort, angioedema, hypotension, dyspnea) as separate events and indicate on the CRF that they are associated with cytokine release syndrome.

Assessment of Severity:

The Investigator will make an assessment of severity for each AE and SAE reported during the study and assign it using the Common Terminology Criteria for Adverse Events (CTCAE) V5.0, which is available at the following location: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf.

An event is defined as 'serious' when it meets ≥ 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality:

- The Investigator is obligated to assess the relationship between study drug and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than that a relationship cannot be ruled out.
- The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study drug administration, will be considered and investigated.
- The Investigator will also consult the Investigator's Brochure in his/her assessment.
- For each AE/SAE, the Investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the Investigator has minimal information to include in the initial report to [redacted] However, the Investigator should always make an assessment of causality for every event before the initial transmission of the SAE data to [redacted]
- The Investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is 1 of the criteria used when determining regulatory reporting requirements.
- All SAEs have to be reported within 24 hours of study site awareness, whether or not considered causally related to the study drug or to a study procedure(s).

Follow-Up of Adverse Events and Serious Adverse Events:

- The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated to fully elucidate the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a patient dies during participation in the study or during a recognized Follow-up Period, the Investigator will provide [redacted] with a copy of any postmortem findings including histopathology.
- New or updated information will be recorded in the originally completed CRF.
- The Investigator will submit any updated SAE data to [redacted] within 24 hours of receipt of new information.
- If a non-SAE becomes serious, this event will be classified as an SAE and the 24-hour reporting clock begins.

10.3.4 Reporting of Serious Adverse Events

Serious Adverse Events Reporting via an Electronic Data Collection Tool:

- The primary mechanism for reporting an SAE will be the electronic data collection tool.
- If the electronic system is unavailable, then the study site will use the paper SAE data collection tool in order to report the event within 24 hours.
- The study site will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given study site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a study site receives a report of a new SAE from a study patient or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the study site can report this information on a paper SAE form or by telephone.

[redacted] (Pharmacovigilance Department) Contact Information:

Location:	United States	Australia
24-hour hotline:	[redacted]	[redacted]
Fax Number:	[redacted]	[redacted]
	[redacted]	

10.4 Appendix 4: Contraceptive Guidance and Collection of Pregnancy Information

Definitions:

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

If fertility is unclear (eg, amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study drug, additional evaluation should be considered.

Women in the following categories are not considered WOCBP:

1. Premenarchal
2. Premenopausal female with 1 of the following:
   - Documented hysterectomy
   - Documented bilateral salpingectomy
   - Documented bilateral oophorectomy

For individuals with permanent infertility due to an alternate medical cause other than the above, (eg, mullerian agenesis, androgen insensitivity), Investigator discretion should be applied to determining study entry.

Note: Documentation can come from the site personnel's: review of the patient's medical records, medical examination, or medical history interview.

3. Postmenopausal female
   - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
   - A high follicle-stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, confirmation with more than 1 FSH measurement is required.
   - Females on HRT and whose menopausal status is in doubt will be required to use 1 of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance:

NL-201 and pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm.

Participants should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study, participants of childbearing potential must adhere to the contraception requirement from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 60 days after the last dose of NL-201 or 120 days after the last dose of pembrolizumab for WOCBP and up to 90 days after the last dose of NL-201 or 120 days after the last dose of pembrolizumab for men. If there is any question that a participant of childbearing potential will not reliably comply with the requirements for contraception, that participant should not be entered into the study.

It is unknown whether pembrolizumab is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, participants who are breastfeeding are not eligible for enrollment.

- Contraceptives[a] allowed during the study include:
  - Highly effective methods[b] that have low user dependency
    Failure rate of < 1% per year when used consistently and correctly.
    - Implantable progestogen-only hormone contraception associated with inhibition of ovulation[c]
    - Intrauterine device
    - Intrauterine hormone-releasing system[c]
    - Bilateral tubal occlusion
    - Azoospermic partner (vasectomized or due to a medical cause)

      Azoospermia is a highly effective contraceptive method provided that the partner is the sole sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used. Spermatogenesis cycle is approximately 90 days.

      Note: documentation of azoospermia for a male patient can come from the study site personnel's review of the patient's medical records, medical examination, or medical history interview.
  - Highly effective methods[b] that are user dependent
    Failure rate of < 1% per year when used consistently and correctly.
    - Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation[c]
      - oral
      - intravaginal
      - transdermal
      - injectable

- Progestogen-only hormone contraception associated with inhibition of ovulation[c]
  - oral
  - injectable
- Sexual abstinence

  Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study intervention. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the patient.

a. Contraceptive use by men or women should be consistent with local regulations regarding the use of contraceptive methods for those participating in clinical studies.
b. Failure rate of < 1% per year when used consistently and correctly. Typical use failure rates differ from those when used consistently and correctly.
c. Male condoms must be used in addition to hormonal contraception. If locally required, in accordance with Clinical Trial Facilitation Group guidelines, acceptable contraceptive methods are limited to those which inhibit ovulation as the primary mode of action.

Note: Periodic abstinence (calendar, symptothermal, and post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea method are not acceptable methods of contraception. Male condom and female condom should not be used together due to risk of failure from friction.

Collection of Pregnancy Information

Male patients with partners who become pregnant

- The Investigator will attempt to collect pregnancy information on any male patient's female partner who becomes pregnant while the male patient is in this study. This applies only to male patients who receive NL-201.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the Sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

Female patients who become pregnant

- The Investigator will collect pregnancy information on any female patient who becomes pregnant while participating in this study. Information will be recorded on the appropriate form and submitted to the Sponsor within 24 hours of learning of a patient's pregnancy.
- The patient will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the patient and the neonate and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

- A spontaneous abortion (occurring at < 22 weeks gestational age) or still birth (occurring at > 22 weeks gestational age) is always considered to be an serious adverse event (SAE) and will be reported as such.
- Any post-study pregnancy-related SAE considered reasonably related to the study drug by the Investigator will be reported to the Sponsor as described in Section 8.4.5 and Section 8.4.6. While the Investigator is not obligated to actively seek this information in former study patients, he or she may learn of an SAE through spontaneous reporting.
- Any female patient who becomes pregnant while participating in the study will discontinue study drug or be withdrawn from the study.

10.5 Appendix 5: Liver Safety: Suggested Actions and Follow-Up Assessments and Study Drug Rechallenge Guidelines

Patients with abnormal hepatic laboratory values (ie, aspartate aminotransferase [AST], alanine aminotransferase [ALT], total bilirubin [TBL]) and/or international normalized ratio (INR) and/or signs/symptoms of hepatitis may meet the criteria for withholding or permanent discontinuation of NL-201, as specified in the Guidance for Industry Drug-Induced Liver Injury: Premarketing Clinical Evaluation, July 2009.

The following stopping and/or withholding rules apply to patients for whom another cause of their changes in liver biomarkers (TBL, INR, and transaminases) has not been identified.

Important alternative causes for elevated AST/ALT and/or TBL values include, but are not limited to:

- hepatobiliary tract disease
- viral hepatitis (eg, hepatitis A/B/C/D/E, Epstein-Barr Virus, cytomegalovirus, herpes simplex virus, varicella, toxoplasmosis, and parvovirus)
- right-sided heart failure, hypotension or any cause of hypoxia to the liver causing ischemia
- exposure to hepatotoxic agents/drugs or hepatotoxins, including herbal and dietary supplements, plants and mushrooms
- heritable disorders causing impaired glucuronidation (eg, Gilbert's syndrome, Crigler-Najjar syndrome) and drugs that inhibit bilirubin glucuronidation (eg, indinavir, atazanavir)
- α-1 antitrypsin deficiency
- alcoholic hepatitis
- autoimmune hepatitis
- Wilson's disease and hemochromatosis
- nonalcoholic fatty liver disease including steatohepatitis
- non-hepatic causes (eg, rhabdomylosis, hemolysis)

Liver Chemistry Stopping Criteria – Liver Stopping Event	
ALT or AST-absolute	alanine aminotransferase (ALT) or aspartate aminotransferase (AST) ≥ 5x the upper limit of normal (ULN)
ALT or AST increase	ALT or AST ≥ 3 x ULN persists for ≥ 4 weeks
Bilirubin[a,b]	ALT or AST ≥ 3 x ULN and total bilirubin ≥ 2 x ULN (> 35% direct bilirubin)
INR[2]	ALT or AST ≥ 3 x ULN and international normalized ratio (INR) > 1.5
Cannot monitor	ALT or AST ≥ 3 x ULN and cannot be monitored weekly for 4 weeks
Symptomatic[c]	ALT or AST ≥ 3 x ULN associated with symptoms (new or worsening) believed to be related to liver injury or hypersensitivity

Required Actions, Monitoring, and Follow up Assessments	
Actions	**Follow-up Assessments**
• **Immediately** discontinue study drug. • Report the event to PPD **within 24 hours** • Complete the reporting form and complete a serious adverse event (SAE) data collection tool if the event also met the criteria for an SAE.[b] • Perform liver chemistry follow-up assessments as described in the Follow-up Assessment column. • Do not restart or rechallenge patient with study drug • Monitor the patient until liver chemistry test abnormalities resolve, stabilize, or return to baseline (see **MONITORING**). **MONITORING:** **If ALT ≥ 3 x ULN AND total bilirubin ≥ 2 x ULN OR INR > 1.5:** • Repeat liver chemistry tests (include ALT, AST, alkaline phosphatase, total bilirubin, and INR) and perform liver event follow-up assessments within 24 hours. • Monitor patient twice weekly until liver chemistry test abnormalities resolve, stabilize, or return to baseline. • A specialist or hepatology consultation is recommended. **If ALT ≥ 3 x ULN AND total bilirubin < 2 x ULN AND INR ≤ 1.5:** • Perform liver chemistry tests (include ALT, AST, alkaline phosphatase, total bilirubin and INR) and perform liver chemistry follow-up assessments within **24 to 72 hours.** • Monitor patients weekly until liver chemistry abnormalities resolve, stabilize, or return to baseline. • **Do not restart/rechallenge** patient with NL-201 unless allowed per protocol and Neoleukin approval is granted	• Viral hepatitis serology[c] • Obtain INR and recheck with each liver chemistry assessment until the transaminases values show downward trend • Obtain blood sample for pharmacokinetic (PK) analysis after the most recent dose[d] • Obtain serum creatine phosphokinase (CPK), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), glutamate dehydrogenase (GLDH) and serum albumin • Fractionate bilirubin, if total bilirubin ≥ 2 xULN • Obtain complete blood count with differential to assess eosinophilia • Record the appearance or worsening of clinical symptoms of liver injury, or hypersensitivity, on [liver event/expedited reporting form] • Record use of concomitant medications (including acetaminophen, herbal remedies, recreational drugs and other over-the-counter medications) • Record alcohol use on the [liver event alcohol intake form]. **If ALT ≥ 3 x ULN AND total bilirubin ≥ 2 x ULN OR INR > 1.5** obtain the following in addition to the assessments listed above**:** • Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG) or gamma globulins. • Serum acetaminophen adduct assay, when available, to assess potential acetaminophen contribution to liver injury in patients with definite or likely acetaminophen use in the preceding week. Liver imaging (ultrasound, magnetic resonance, or computerized tomography) and/or liver biopsy to evaluate liver disease • Liver biopsy may be considered and discussed with local specialists if available for instance: o In patients when serology raises the possibility of autoimmune hepatitis (AIH) o In patients when suspected drug-induced liver injury (DILI) progresses or fails to resolve on withdrawal of study drug o In patients with acute or chronic atypical presentation.

a Serum bilirubin fractionation should be performed if testing is available. If serum bilirubin fractionation is not immediately available, discontinue study intervention if ALT or AST ≥ 3 x ULN and total bilirubin ≥ 2 x ULN. Additionally, if serum bilirubin fractionation testing is unavailable, record the absence/presence of detectable urinary bilirubin on dipstick which is indicative of direct bilirubin elevations suggesting liver injury.

b All events of ALT or AST ≥ 3 x ULN and total bilirubin ≥ 2 x ULN (> 35% direct bilirubin) or ALT or AST ≥ 3 x ULN and INR >1.5 may indicate severe liver injury (possible 'Hy's Law') and must be reported to Sponsor in an expedited manner and as an SAE if SAE criteria met (excluding studies of hepatic impairment or cirrhosis). The INR stated threshold value will not apply to patients receiving anticoagulants.

c New or worsening symptoms believed to be related to liver injury (such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, or jaundice) or hypersensitivity (such as fever, rash or eosinophilia). d PK sample may not be required for patients known to be receiving placebo or non-comparator interventions. Record the date/time of the PK blood sample draw and the date/time of the last dose of study drug prior to the PK blood sample draw on the CRF. If the date or time of the last dose is unclear, provide the patient's best approximation. If the date/time of the last dose cannot be approximated OR a PK sample cannot be collected in the time period indicated above, do not obtain a PK sample. Instructions for sample handling and shipping are in the Laboratory Manual.

10.6 Appendix 6: Tumor Assessments

10.6.1 Methods of Lesion Measurement

The following considerations are to be made when evaluating the tumor (applicable for Response Evaluation Criteria in Solid Tumors [RECIST] 1.1 and RECIST for use in cancer immunotherapy trials [iRECIST]):

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

Computed tomography (CT) is the best currently available and reproducible method to measure lesions selected for response assessment. Magnetic resonance imaging (MRI) is also acceptable in certain situations. Therefore, imaging of chest, abdomen, pelvis and brain will be performed by CT or MRI in this study.

Bone metastases will be assessed by X-ray or CT or MRI or bone scan in this study.

Cytology, histology: the cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment can be considered if the measurable tumor has met criteria for response or stable disease (SD) in order to differentiate between response (or SD) and progressive disease.

10.6.2 Tumor Assessments According to RECIST 1.1

Categorization of tumor lesions

Measurable disease is defined as the presence of at least 1 measurable lesion. A measurable lesion is a lesion that can be accurately measured in at least 1 dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of 10 mm by CT/MRI scan. Measurability of lesions on CT/MRI scan is defined based on the assumption that CT/MRI slice thickness is 5 mm or less. However, when CT/MRI scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness.

Note: methods of tumor assessments as outlined in RECIST 1.1 are slightly modified in this study in order to achieve a better standardization: images of chest, abdomen, pelvis and brain should be performed by CT or MRI and bone lesions will be assessed by X-ray or CT or MRI or bone scan. Clinical exam or chest X-ray is not regarded as appropriate methods for tumor assessment in this study.

Non-measurable lesions are all other lesions, including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT/MRI scan (CT/MRI scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft tissue component meets the definition of measurability described above.

Blastic bone lesions are non-measurable. Bone scan, positron emission tomography (PET) scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.

Cystic lesions: lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions neither measurable nor non-measurable since they are, by definition, simple cysts. 'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions that are situated in a previously irradiated area, or in an area patient to other loco-regional therapy, should be considered non-measurable lesions and will be evaluated only qualitatively as non-target lesions.

Baseline documentation of target and non-target lesions

When more than 1 measurable lesion is present at baseline all lesions up to a maximum of 5 lesions total (and a maximum of 2 lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline (this means in instances where patients have only 1 or 2 organ sites involved a maximum of 2 and 4 lesions respectively will be recorded).

Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements.

Lymph nodes merit special mention since they are normal anatomical structures which may be visible by imaging even if not involved by tumor. As mentioned above pathological nodes which are defined as measurable and may be identified as target lesions must meet the criterion of a short axis of ≥ 15 mm by CT/MRI scan. Only the short axis of these nodes will contribute to the baseline sum. All other pathological nodes (those with short axis ≥ 10 mm but < 15 mm) should be considered non-target lesions. Nodes that have a short axis < 10 mm are considered non-pathological and should not be recorded or followed.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes will be

included in the sum, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

All other lesions (or sites of disease) including pathological lymph nodes with short axis ≥ 10 mm but < 15 mm should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as 'present', 'absent', or in rare cases 'unequivocal progression' (more details to follow). In addition, it is possible to record multiple non-target lesions involving the same organ as a single item on the case record form (eg, 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

Evaluation of target, non-target and new lesions according to RECIST 1.1

All images will be interpreted (assessment of response) by the radiologist according to RECIST 1.1. At each tumor assessment visit, response is assessed first separately for target, non-target lesions (see Table 17) and new lesions (see below "appearance of new lesions"). These evaluations are then used to calculate the overall lesion response considering target lesions, non-target lesions and new lesions for patients with measurable disease (see Table 18) and non-target lesions and new lesions for patients with non-measurable disease only at baseline (see Table 19).

Table 17 Assessment of Response According to RECIST 1.1

Response Category for Target Lesions	
CR	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.
PR	At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters of the diameters of target lesions.
SD	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.
PD	At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm.
Response Category for Non-Target Lesions	
CR	Disappearance of all non-target lesions. All lymph nodes must be non-pathological in size (< 10 mm short axis).
Non-CR/Non-PD	Persistence of 1 or more non-target lesions
PD	Unequivocal progression (see comments below) of existing non-target lesions.

CR = complete response; PD = progressive disease; PR = partial response; SD = stable disease

Appearance of new lesions

The appearance of any new lesions is considered progression. There are no specific criteria for the identification of new radiographic lesions; however, the finding of a new lesion should be unequivocal: ie, not attributable to differences in scanning technique, change in imaging modality or findings thought to represent something other than tumor (for example, some 'new'

bone lesions may be simply healing or flare of pre-existing lesions). This is particularly important when the patient's baseline lesions show partial or CR. For example, necrosis of a liver lesion may be reported on a CT/MRI scan report as a 'new' cystic lesion, which it is not. A lesion identified on a follow-up study in an anatomical location that was not scanned at baseline is considered a new lesion and will indicate disease progression. An example of this is the patient who has visceral disease at baseline and while on study has a CT or MRI brain ordered which reveals metastases. The patient's brain metastases are considered to be evidence of disease progression even if he/she did not have brain imaging at baseline. If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

Special notes on assessment of progression of non-target disease

When the patient also has measurable disease: In this setting, to achieve 'unequivocal progression' on the basis of the non-target disease, there must be an overall level of substantial worsening in non-target disease such that, even in presence of SD or PR in target disease, the overall tumor burden has increased sufficiently to merit discontinuation of therapy. A modest 'increase' in the size of 1 or more non-target lesions is usually not sufficient to quality for unequivocal progression status. The designation of overall progression solely on the basis of change in non-target disease in the face of SD or PR of target disease will therefore be extremely rare.

When the patient has only non-measurable disease: The same general concepts apply here as noted above, however, in this instance there is no measurable disease assessment to factor into the interpretation of an increase in non-measurable disease burden. Because worsening in non-target disease cannot be easily quantified (by definition: if all lesions are truly non-measurable) a useful test that can be applied when assessing patients for unequivocal progression is to consider if the increase in overall disease burden based on the change in non-measurable disease is comparable in magnitude to the increase that would be required to declare disease progression for measurable disease: ie, an increase in tumor burden representing an additional 73% increase in 'volume' (which is equivalent to a 20% increase diameter in a measurable lesion). Examples include an increase in a pleural effusion from 'trace' to 'large', an increase in lymphangitic disease from localized to widespread, or may be described as 'sufficient to require a change in therapy'. If 'unequivocal progression' is seen, the patient should be considered to have had overall disease progression at that point. While it would be ideal to have objective criteria to apply to non-measurable disease, the very nature of that disease makes it impossible to do so; therefore, the increase must be substantial.

Evaluation of overall lesion response according to RECIST 1.1

Only patients with at least 1 measurable lesion or non-measurable disease only at baseline (enrollment not allowed according to protocol) will be included in the tumor assessment according to RECIST 1.1. For patients with measurable disease at baseline the evaluation of overall response at each assessment is a composite of the target lesion response, non-target lesion response and presence of new lesions as shown below in Table 18 (ie, target lesions with/without

non-target lesions and new lesions). For patients with non-measurable disease only at baseline the evaluation of overall response at each assessment is a composite of the non-target lesion response and presence of new lesions as shown below in Table 19 (ie, non-target lesions only and new lesions). Patients with symptoms of rapidly progressing disease without radiologic evidence will be classified as progression only when clear evidence of clinical deterioration is available and patient is prematurely withdrawn due to 'disease progression'. In order for SD to be assigned, patients' lesions must meet the SD criteria at least once after 6 weeks after study entry.

Table 18 Overall Lesion Response at Each Assessment: Patients With Target Lesions (± Non-Target Lesions)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not evaluated	No	PR
PR	CR	No	PR
PR	Non-PD	No	PR
PR	Not all evaluated	No	PR
SD	CR	No	SD
SD	Non-PD	No	SD
SD	Not all evaluated	No	SD
Not all evaluated	CR/Non-PD/Not all evaluated	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR = complete response; NE = not evaluable; PD = progressive disease; PR = partial response; SD = stable disease

Table 19 Overall Lesion Response at Each Assessment: Patients With Non-Target Lesions Only

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
-	CR	No	CR
-	Non-CR/Non-PD	No	Non-CR/Non-PD
-	Not all evaluated	No	NE
-	Unequivocal PD	Yes or No	PD
-	Any	Yes	PD

CR = complete response; NE = not evaluable; PD = progressive disease; SD = stable disease

Evaluation of lesions and assessment of overall response following on-study interventions

Following any on-study intervention (eg, local radiation, tumor surgery, paracentesis of ascites, pleural effusions, or chemoembolization) the Investigator should continue assessing target lesions quantitatively, non-target lesions qualitatively, and reporting any new lesions. As a general rule, overall response following local interventions will be limited to disease progression

or "NE" (not evaluable) in case criteria for disease progression are not met. However, the Investigator should take into consideration the reason for the on-study intervention (eg, progression of a lesion or shrinkage of a lesion) when providing the overall assessment.

10.6.3 iRECIST Response Assessment

Overall response will also be assessed using iRECIST. Immunotherapeutics may result in infiltration of immune cells leading to transient increase in the size in malignant lesions, or undetectable lesions becoming detectable. The criteria are identical to those of RECIST 1.1 in many respects but have been adapted to account for instances where an increase in tumor burden, or the appearance of new lesions, does not reflect true tumor progression.

Key differences are described below. All responses defined using iRECIST criteria are designated with a prefix. iRECIST time-point and best overall responses will be recorded separately.

Confirming Progression

Unlike RECIST 1.1, iRECIST requires the confirmation of progression and uses the terms immune unconfirmed progressive disease (iUPD) (unconfirmed progression) and immune confirmed progressive disease (iCPD) (confirmed progression). Confirmatory scans should be performed at least 4 weeks, but no longer than 8 weeks after iUPD.

iCPD is confirmed if further increase in tumor burden, compared to the last assessment, is seen as evidenced by 1 or more of the following:

- Continued increase in tumor burden (from iUPD) where RECIST 1.1 definitions of progression had been met (from nadir) in target, non-target disease or new lesions
  - Progression in target disease worsens with an increase of at least 5 mm in the absolute value of the sum
  - Continued unequivocal progression in non-target disease with an increase in tumor burden
  - Increase in size of previously identified new lesion (s) (an increase of at least 5 mm in the absolute value of the sum of those considered to be target new lesions) or additional new lesions.
- RECIST 1.1 criteria are met in lesions types (target or non-target or new lesions) where progression was not previously identified, including the appearance of additional new lesions.

If iUPD is not confirmed at the next assessment, then the appropriate response will be assigned (iUPD if the criteria are still met, but no worsening, or immune stable disease [iSD], immune partial response [iPR] or immune complete response [iCR] if those criteria are met compared to baseline). As can be seen in Table 20, the prior documentation of iUPD does not preclude assigning iCR, iPR, or iSD in subsequent time-point assessments or as best overall response (BOR) providing that iCPD is not documented at the next assessment after iUPD.

New Lesions

New lesions should be assessed and measured as they appear using RECIST 1.1 criteria (maximum of 5 lesions, no more than 2 per site, at least 10 mm in long axis (or 15 mm in short axis for nodal lesions), and recorded as New Lesions-Target (NLT) and New Lesion-Non-Target (NLNT) to allow clear differentiation from baseline target and non-target lesions.

New lesions may either meet the criteria of NLT or NLNT to drive iUPD (or iCPD). However, the measurements of target lesions should NOT be included in the sum of measures of original target lesions identified at baseline. Rather, these measurements will be collected on a separate table in the case record form.

PD is confirmed in the New Lesion category if the next imaging assessment, conducted at least 4 weeks (but not more than 8 weeks) after iUPD confirms further progression from iUPD with either an increase of at least 5 mm in the absolute value of the sum of NLT OR an increase (but not necessarily unequivocal increase) in the size of NLNT lesions OR the appearance of additional new lesions.

Table 20 Time Point (TP) iResponse

Target lesions[a]	Non-target lesions[a]	New lesions[a]	Time point response	
			No prior iUPD[b]	Prior iUPD[b,c]
iCR	iCR	No	iCR	iCR
iCR	Non-iCR/ Non-iUPD	No	iPR	iPR
iPR	Non-iCR/ Non-iUPD	No	iPR	iPR
iSD	Non-iCR/ Non-iUPD	No	iSD	iSD
iUPD with no change OR decrease from last TP	iUPD with no change OR decrease from last TP	Yes	NA	NLs confirms iCPD if NLs were previously identified and increase in size (≥ 5 mm in SOM for NLT or any increase for NLNT) or number. If no change in NLs (size or number) from last TP, remains iUPD.
iSD	iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based in further increase in size of NT disease (need not meet RECIST 1.1 criteria for unequivocal PD)
iUPD	Non-iCR/ Non-iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on further increase in SOM of at least 5 mm, otherwise remains iUPD
iUPD	iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on further increase in: * previously identified T lesion iUPD SOM ≥ 5 mm and/or * NT lesion iUPD (prior assessment - need not be unequivocal PD
iUPD	iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on further increase in: * previously identified T lesion iUPD ≥ 5 mm and/or * previously identified NT lesion iUPD (need not be unequivocal) and/or * size or number of new lesions previously identified
Non-iUPD/ PD	Non-iUPD/ PD	Yes	iUPD	Remains iUPD unless iCPD confirmed based on increase in size or number of new lesions previously identified

CR = complete response; iCPD = immune confirmed progressive disease; iCR = immune complete response; iPR = immune partial response; iRECIST = RECIST for use in cancer immunotherapy trials; iSD = immune stable disease; iUPD: immune unconfirmed progressive disease; NL = new lesion; NLNT = new lesion-non-target; NLT = new lesion-target; PD = progressive disease; PR = partial response; RECIST = Response Evaluation Criteria in Solid Tumors; SD = stable disease; SOM = sum of measures; TP = time point

a Using RECIST 1.1 principles. If no pseudoprogression (PSPD) occurs, RECIST 1.1 and iRECIST categories for CR, PR and SD would be the same.

b In any lesion category.

c Previously identified in assessment immediately prior to this TP.

All patients will have their immune best overall response (iBOR) from the start of study treatment until the end of treatment classified as outlined in Table 21.

Table 21 iRECIST Best Overall Response (iBOR)

TPR1	TPR2	TPR3	TPR4	TPR5	iBOR
iCR	iCR, iPR, iUPD, NE	iCR, iPR, iUPD, NE	iUPD	iUPD	iCR
iUPD	iPR, iSD, NE	iCR	iCR, iPR, iSD, iUPD, NE	iCR, iPR, iSD, iUPD, iCPD, NE	iCR
iUPD	iPR, iSD, NE	iPR, iSD, iUPD, NE	iPR, iSD, iUPD, NE, iCPD	iPR, iSD, iUPD, NE, iCPD	iPR
iUPD	iSD, NE	PR	iPR, iSD, iUPD, NE	iPR, iSD, iUPD, iCPD, NE	iPR
iUPD	iSD	iSD, iUPD, NE	iSD, iUPD, iCPD, NE	iSD, iUPD, ICPD, NE	iSD
iUPD	iCPD	Anything	Anything	Anything	iCPD
iUPD	iUPD	iCPD	Anything	Anything	iCPD
iUPD	NE	NE	NE	NE	iUPD

CR = complete response; iBOR = immune best overall response; iCPD = immune confirmed disease progression; iCR = immune complete response; iPR = immune partial response; iSD = immune stable disease; iUPD = immune unconfirmed progressive disease; NE = not evaluable that cycle; PD = progressive disease; PR = partial response; TPR = response at that time point

NOTES:

Table assumes a randomized study where confirmation of CR or PR is not required.

Designation "I" for BOR can be used to indicate prior iUPD to aid in data interpretation.

For patients with non-target disease only at baseline, only CR or non-CR/non-PD can be assigned at each TPR but is not shown in the table for ease of presentation.

Source: Seymour 2017[31]

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12 SUMMARY OF CHANGES

Version 2.0 (26 August 2020) replaces Version 1.0 (29 July 2020)

Description and rationale for changes:

- The starting dose has been changed from 0.3 to 0.1 µg/kg.
  A first-in-human starting dose of 0.1 µg/kg for NL-201 is anticipated to approximate the lowest pharmacologically active dose (PAD). This dose is based on the minimum anticipated biologic effect level approach using results from the most sensitive *in vitro* assays. In addition, a starting dose of 0.1 µg/kg for NL-201 is 150-fold lower than the no observed adverse effect level (NOAEL) of 15 µg/kg (50-fold lower than the human equivalent dose of 5 µg/kg) in monkeys when administered weekly for 5 consecutive weeks.
- In Section 9.3 (Population for Analyses), the "DLT-Evaluable Population" has been added.
  This population was defined in body of protocol but was missing from statistical considerations.
- Administrative changes have been throughout the protocol (version number and approval date).

Amendment 1.1, Version 2.1 (04 December 2020) replaces version 2.0 (26 August 2020)

Description and rationale for changes:

- The first-in-human starting dose rationale has been updated.
- Administrative changes have been throughout the protocol (version number and approval date).

Amendment 2.0 (23 February 2021) replaces Amendment 1.1, version 2.1 (04 December 2020)

Description and rationale for changes:

Description of Change	Rationale for Change	Location(s) for Change
Sponsor Address	The Sponsor's address was updated to: 188 E Blaine Street Suite 450 Seattle, WA 98102	Title page
The Regulatory Agency Identifier Number was added.	NCT: 04659629 was previously not available, and was added to this amendment.	Title page
Respiration rate was added to vital signs (Section 8.2).	This is a standard measurement of vital signs.	Section 8.3.2
The schedule of events and overall design sections have been updated with additional descriptions that biopsy samples will be collected from a minimum of 8 patients.	The changes were made to make the protocol consistent.	Tables 1 through 4, and Section 4.1
The timing of pregnancy testing has been updated in the schedule of events (Table 1 Schedule A [Q3W Dosing]; Table 2 Schedule B [D1 & D8 Dosing]; Table 3 Schedule A [Q3W Dosing if Step-dosing is Implemented]; and Table 4 Schedule B [D1 & D8 if Step-dosing is Implemented]), and in inclusion criterion #8. Both serum and urine pregnancy testing is acceptable.	The timing of pregnancy testing and appropriates samples were clarified.	Tables 1 through 4, and inclusion criterion #8
Pharmacodynamic blood sampling is not required at the End of Treatment visit for Schedule B (Table 2) and was removed.	This change corrected a transcription error.	Table 2
Hematology and chemistry samples were not collected on Schedule B (step dosing, Table 4) at the radiologic follow-up visit	This change was made to remove unnecessary samples.	Table 4
Exclusion Criterion # 20 'signing of consent' was replaced with 'first planned dose'	The updated wording clarifies prior/concurrent clinical study experience.	Section 5.2
Wording for administration in Section 6.1.1 (in-text table) was updated to cross-reference the Pharmacy Instructions	The updated wording provides the correct reference to the resource that describes the process for preparing the drug substance.	Section 6.1.1

Description of Change	Rationale for Change	Location(s) for Change
The dose and frequency of concomitant medications will be collected for concomitant medications of interest only (Section 6.5), but only when they become available.	Additional information regarding concomitant medications of interest will better inform the Sponsor.	Section 6.5
ECOG Performance Status will be collected at Screening, Day 1 of all cycles, and the End of Treatment visit for all schedules (Tables 1 through 4)	This change was made to capture the data collected in inclusion criterion #4.	Tables 1 through 4
The CT scanning was updated to match the occurrence in per Table 1, Schedule A (Q3W Dosing)	The change was made to resolve a transcription error.	Table 2
Viral serology (Table 2 Schedule B [D1 & D8 Dosing]) was updated to match the wording in Schedule A (Table 2)	This change was made to resolve a transcription error.	Table 2
NOAEL was updated to match Section 4.4.	The NOAEL was updated to 11.44 µg/kg to make with consistent throughout the document.	Section 2.2.2
For screen failures, medical history and prior therapies were removed (Section 5.3)	This data is not necessary to collect on screen failures.	Section 5.3
'Biopsy-related adverse events' were removed from the first sentence.	The change was made to clarify the risks.	Section 2.3
The risk table (Section 2.3.1) was updated: The row 'Biopsy related adverse event' was removed.	This is no longer needed.	Section 2.3.1
The row 'Public health related exposures during global pandemics' was updated with additional wording to clarify the timing of SARS-CoV-2 immunization(s) relative to the timing of the first dose of study drug on Cycle 1 Day 1 of any schedule. The first bullet point was removed ('Non-dosing days beyond C1D2 requiring sample collection can be performed by home healthcare provider as an alternative to a site visit.'). The previously fifth bullet point was reworded ('Vaccination approved for the prevention of infection are allowed'): 'Vaccination approved for the prevention of infection are allowed if completed at least 14 days before Cycle 1 Day 1 or any time after Cycle 2 Day 1.' A new fifth bullet point was added ('Severe acute respiratory coronavirus 2 (SARS-CoV-2) testing will be required as per local regulations').	Additional wording was included to inform patients on when to consider receiving a SARS-CoV-2 vaccine while on study.	Section 2.3.1

Description of Change	Rationale for Change	Location(s) for Change
A new sixth bullet point was added ('An enrolled patient may have a study drug (NL-201) dosing delay of up to 14 days, if the patient received an approved SARS-CoV-2 immunization or treatment before receiving their first dose of study drug on C1D1 in any schedule.').		
The number of patients was updated to 'up to approximately n = 60' in Part 1 and 'n=60' for Part 2 of Figure 1-1 (Overall Study Design).	In the previous protocol amendment, the maximum number of patients was absent from this figure for Part 1. Part 1 was updated to enroll 60 patients for each Schedule A and Schedule B in cohorts of n ≥ 3 patients for a maximum of 60 patients in total. In the previous protocol amendment, the maximum number of patients was 30. Part 2 was updated to enroll 30 patients in each cohort, up to a maximum of 60 patients in total. Combined enrollment in Part 1 and Part 2 will be 120 patients.	Figure 1
The last bullet point was updated to remove the following wording; 'in the CRF'.	The change was made to clarify that laboratory values will be recorded, but not in the CRF.	Section 8.3.4
Several instances of 'Pharmacy Manual' were changed to 'Pharmacy Instructions'	The change was made to clarify that the Pharmacy Instructions should be referred to.	Sections 6, 6.1.1, and 6.1.2
'Pharmacy Manual' was changed to 'Laboratory Manual' in the footnote c in the table pertaining to liver chemistry stopping criteria.	The change was made to clarify that the Laboratory Manual should be referred to.	Appendix 10.5
The definition of dose limiting toxicities was updated with the addition of 2 definitions, which are: 'recurrent Grade 3 infusion-related reaction despite pre-medication. Grade 4 infusion-related reaction on the first occurrence.' 'dose delay of > 14 days for study drug due to an adverse event'	The DLT definition was updated to align with current practice and to align with FDA comments on 17 December 2020.	Section 6.2.1.2
The last bullet point was updated to: 'Concomitant therapies of interest only and if available during the course of the study: dosage information including dose and frequency.'	The updated wording clarifies concomitant therapies of interest.	Section 6.5
The addition of 'PK characterization will assess both the NL-201 and the free protein'	Additional wording clarifies the PK characterization of NL-201 and aligns with FDA comments on 07 January 2020.	Section 8.6

Description of Change	Rationale for Change	Location(s) for Change
The addition of 'start of new anti-cancer therapy, withdrawal of consent, study closure, or death, whichever occurs first' was added to the overall design.	The wording was updated for additional consistency.	Synopsis, Tables 1 through 4, and Section 4.1
The minimum number of biopsies has been changed from 10 to 16 patients.	The change was made to reflect the updated number of biopsies to be conducted in Part 2.	Sections 1.1 and 4.1
In the second sentence of the last paragraph, '≤ 5%' was replaced with '≤ 2.5%'. In the fourth sentence of the last paragraph, '≤' was removed and 'objective responses' was added after '0/18'.	This change was made to resolve a transcription error.	Section 9.2
'(screening only)' was added to Hemoglobin A1c.	The updated wording clarifies the timing of this test.	Table 10
The 6th bullet under 'The Investigator must assign the following AE attributes' was updated. 'Action taken. If adverse event severity changes, record each change as a single event.'	The wording clarifies the recording of an adverse event.	Section 10.3.3

Amendment 3.0 (26 August 2021) replaces Amendment 2.0, version 2.1 (23 February 2021)

Description and Rationale for Changes:

Description of Change	Rationale for Change	Location(s) for Change
Updates to the Schedule of Events tables	• Clarified timing for Eastern Cooperative Oncology Group (ECOG) performance status • Clarified timing of Pregnancy test • Clarified timing of safety laboratory tests • Clarified timing of Day 8 for collection of PK samples	Schedule of Events, Tables 1 through 4
Updates to the Inclusion and Exclusion Criteria	• Broaden autoimmune disease eligibility requirements. • Lowering hemoglobin from 9.0 to 8.0 g/dL and remove transfusion window. • Changing steroid limit from ≤ 7.5 to ≤ 10 mg. • Calculation for renal function set to Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI). • Clarified timing if pregnancy test.	Sections 5.1 and 5.2
Risk assessment table updated	• Clarification of management of cytokine release syndrome and capillary leak syndrome per investigators discretions and according to institutional guidelines. • Summary of Data/Rationale for Risk for Auto-immunity updated.	Table 5
Capillary leak syndrome added to the Dose Delay and Modification Guideline table	• Capillary leak syndrome added as a new potential toxicity.	Table 9
Requirement to re-confirm eligibility on Cycle 1 Day 1	• Enrollment language in protocol has been clarified to specify the safety laboratory tests will be reconfirmed before dosing.	Schedule of Events, Tables 1 through 4, and Section 8
Definition of “enrolled” updated.	• Definition updated to include patients that have met eligibility criteria and to receive drug.	Section 9.2
Retention of source data and samples updated to ≥ 25 years or per local agency requirements	• Updated to align with Health Canada requirement	Section 10.1.6
Add albumin to the list of clinical laboratory tests	• Albumin is followed because it can change with the development of vascular leak syndrome.	Table 11

Description of Change	Rationale for Change	Location(s) for Change
Clarification and typographical errors	• Bioassays collection of serum, rather than plasma • Formatting of document to align with Style Guide including paragraphs, tables and figures etc.	General

Amendment 4.0 (03 December 2021) replaces Amendment 3.0 (26 August 2021)

Description and Rationale for Changes:

Description of Change	Rationale for Change	Location(s) for Change
Signatory updates	• Change of signatories	Sponsor Signatory page
Addition of Intermediate Doses	• At the recommendation of the DMC, predetermined intermediate dose levels may be tested.	Synopsis, Schema, Section 6.2.1.1, and Table 7
Risk Assessment table updated	• Inclusion of "infusion-related reaction" to the first Potential Risk of Clinical Significance. In addition to the specific risks described below, unknown risks may be associated with a first in human application (eg, allergic reactions). • Specific PTs have been added to clarify the constellation of symptoms which are expected as part of the general PTs.	Table 5
mTPI design updated	• Updated to align with presentation style in literature.	Figure 3
Determination of MTD in Part 1 updated	• Updated to clarify the MTD and RP2D determination.	Section 9.4.2
Formatting	• Formatting of document to align with Style Guide.	General

Amendment 5.0 (26 January 2022) replaces Amendment 4.0 (03 December 2021)

Description and Rationale for Changes:

Description of Change	Rationale for Change	Location(s) for Change
Addition of Parts 3 and 4 - NL-201 in combination with pembrolizumab	• It is hypothesized that NL-201 in combination with pembrolizumab may improve response to pembrolizumab as was demonstrated in animal models. NL-201 has been shown to increase PD-1 expression in CD+ T-cells, increased the immune infiltration of the TME, and may make tumor cells more sensitive to the effects of pembrolizumab.	Synopsis, Schema, Schedule of Events, Sections 2.2.3, 2.3.1.1, 2.3.1.2, 2.3.2, 3, 4.1, 4.3, 5, 6.1.1, 6.1.4, 6.2.1.2, 6.2.1.3, 6.2.2, 6.5, 6.6, 7.1, 8.1.3, 8.4.2, 8.4.6, 8.5, 8.7.3, 9, Appendix 2, Appendix 4
Note for AE review and Concomitant medication review updated	• Note updated to clarify SAEs related to standard laboratory tests	Schedule of Events,
CT Scan clarification	• Clarification of 4-week CT scan to confirm PR or CR as per RECIST criteria	Schedule of Events, Section 8.2.1
Risk Assessment table updated	• Required updates as a result of changes in Table 11	Table 5
Pre-Medication modifications	• Addition of pre-medication requirement at C1D1 for dose levels > 1 µg/kg of NL-201	Section 6.1.3
Dose Modification table updated	• To modify guidance for specific potential toxicities that are manageable with supportive measures by the investigator, eg, cytokine release syndrome	Table 11
Publication policy updated	• Clarification that site's publication contract provisions supersedes the protocol publication policy	Section 10.1.11
Formatting	• Formatting of document to align with Style Guide.	General

Amendment 6.0 (08 September 2022) replaces Amendment 5.0 (26 January 2022)

Description and Rationale for Changes:

Description of Change	Rationale for Change	Location(s) for Change
Protocol Title updated to include combination therapy with pembrolizumab	Requested by HREC	Throughout protocol
Part 3 cohort number corrected	• Correction of typographical error	Synopsis, Table 9
Removing NL-201 dose levels 18 and 24 µg/kg	• Not likely to achieve higher end of the initial dose range.	Synopsis, Table 8 and 9, Figure 1 and 3
Addition of language for intrasubject dose escalation upon clearance of each dose level for Part 1	• To allow patients to benefit from higher doses (safely cleared by the DMC)	Synopsis, Section 4.1, Section 6.2.1
Study Schema Part 1 and Part 3 updated	• Updated to reflect changes made in the protocol	Figure 1
Included language to allow for steroid medication	• Allow for circumstances where steroid medication is needed	Section 6.1.4
Hematology and chemistry sampling added on Schedule B (step dosing, Table 4)	• Inadvertently left out of protocol for hematology and chemistry at end of treatment (EOT)	Schedule of Events (Table 4)
De-couple Schedule A and Schedule B dose escalation for Part 1	• Schedule B dose escalation no longer gated on clearance of dose level in Schedule A	Synopsis and Section 4.1
Note on concomitant medication review updated	• Note updated to clarify collection of concomitant medications	Schedule of Events
Clarification added to weight assessment note in Schedule of Events for weight adjustment and dose calculation	• Updated to reflect the protocol	Schedule of Events
Addition to backfill cleared dose levels in Part 1	• To collect additional data for the evaluation of including, but not limited to, pre-medications, biopsy and antitumor activity.	Synopsis, Sections 4.1, Section 4.1.2, Section 4.1.3, Section 9.2

Description of Change	Rationale for Change	Location(s) for Change
Addition of optional biopsies in Part 1	• Generate anecdotal data on impact to tumor microenvironment	Synopsis, Schedule of Events (Table 1 and 2), Section 4.1
Addition of language around risk of pembrolizumab administration	• Requested by HREC	Section 2.3.1.2
Addition toxicity mitigations, such as steroids	• Based on Administrative Letters 2 through 5	Section 6.1.3
Updated Part 2 target population	• Incomplete sentence	Section 5
Updated observation time after first new patient of each new dose level to 48 hours	• Most of the adverse events are recorded within first 48 hours	Synopsis, Section 4.1,
Included thrombus prophylaxis as acceptable to exclusion criteria # 7	• For thrombosis that is ongoing and being treated, these patients are suitable to come onto study.	Section 5.2
Under Section 10.3.1, Definition of Adverse Event, Events Meeting the Adverse Event Definition: Removed - "For situations when an AE or SAE is due to the primary tumor type being studied, all known signs and symptoms should be reported. Death due to disease progression in the absence of signs and symptoms should be reported as the primary tumor type (eg, RCC). Note: The term "disease progression" should not be used to describe the AE".	• Clarification on classification of signs, symptoms or deaths related to disease progression • Natural progression or deterioration of the patient's malignancy/disorder under study (including new sites of metastasis and death due to disease progression) will be recorded as part of the efficacy evaluation and should not be reported as an AE or as an SAE; unless, signs or symptoms of the primary tumor type are considered more severe than expected for the patient's condition.	Section 10.3.1
Addition that death due to disease progression will not be recorded as a SAE	• Updated to align with Administration Letter 5	Section 10.3.3

Description of Change	Rationale for Change	Location(s) for Change
Interventions for NL-201 monotherapy and pembrolizumab combination therapy	• Instituting interventions to mitigate the risks for hypotension and cytokine-related symptoms that have been observed in patients on the monotherapy arm of NL-201-101	Schedule of Events and Section 6.1.3
Footnote updated for dosing table to clarify dose expansion and cohort expansion	• Updated to reflect protocol	Synopsis, Table 8 and 9
Number of patients for Part 1 and overall number of patients for study	• Updated to reflect protocol	Synopsis, Section 4.1, Section 4.1.2, Section 9.2
Dosing instructions for pembrolizumab	• Updated to clarify that pembrolizumab must always be administered on the same day as NL-201	Table 7
Collection of PK and cytokines timepoints clarified	• Clarification to the SOE regarding collection for PK and cytokines (not relevant to other sample draws) to state that the collection timepoints on D1 (eg, pre and post EOI) should be anchored to the infusion of NL-201 and NOT to the infusion of pembrolizumab.	Schedule of Events
Language added for pembrolizumab risks	• Requested by HREC	Section 2.3.1
Update to number of sites	• Current estimation	Section 4.1.4
Clarification added for when scans after disease progression can be performed	• Updated to reflect protocol	Section 6.1.1.1
Clarification added to delays and doses skipped with regard to Cycle number	• To clarify that Cycle 2 sampling is still required if Cycle 2 is skipped	Section 6.2.2.1
Language added to the assessment of NL-201 dependent effects on tumor microenvironment	• To understand the relationship between the biological characteristics of tumors before treatment and patient outcomes	Section 8.7.2

Description of Change	Rationale for Change	Location(s) for Change
Clarification on timing of tumor biopsies for Schedule A and Schedule B	• Updated to reflect protocol	Schedule of Events, Synopsis, Section 4.1
Clarification on end of treatment visit	• Updated to reflect protocol	Synopsis, Section 4.1, Section 8.1.3
Updated men's contraceptive inclusion criteria #9	• To align per CTFG contraception guidance	Section 5.1, Section 10.4
Updated overdose reporting language	• Updated to reflect protocol	Section 8.5
Updated adverse events and serious adverse event section with instructions for reporting cytokine release syndrome	• Updated to reflect protocol	Section 10.3.3
Removal of subsequent dosing for outpatient setting in Part 3	• Subsequent dosing guidance is described in Section 6.2.2	Synopsis, Section 4.1, Section 6.2.2
Update of radiologic follow up language	• During dose escalation to be less burdensome to patients.	Synopsis, Schedule of Events, Section 4.1, Section 8.1.2, Section 8.1.4, Section 8.2.1
Update on long term follow-up	• Adequate data can be collected to assess endpoints with less the frequent assessments	Synopsis, Schedule of Events, Section 4.1, Section 8.1.2, Section 8.1.5, Section 8.2.1
Addition of language for cytokine release syndrome treatment guidance	• Additional guidance for a known toxicity	Section 6.1.4
Signatory updates	• Change in signatories	Sponsor Signature page
Formatting	• Formatting of document to align with Style Guide.	General