A phase 1b/2a, open label study to evaluate anti-tumor efficacy and safety of rhIL-7-hyFc (NT-I7) in combination with anti-PD-L1 (atezolizumab) in patients with anti-PD-1/PD-L1 naïve or relapsed/refractory high-risk skin cancers

NCT03901573

19 January 2023

Note: Signatures have been redacted from this document.

Protocol Title: A phase 1b/2a, open label study to evaluate anti-tumor efficacy and safety of rhIL-7-hyFc (NT-I7) in combination with anti-PD-L1 (atezolizumab) in patients with anti-PD-1/PD-L1 naïve or relapsed/refractory high-risk skin cancers

Protocol Number:	NIT-106 (ION-02)
Version Date:	Amendment 5_v1.0_19JAN2023
Products:	Atezolizumab (MPDL3280A; NSC 783608) NT-I7 (rhIL-7-hyFc)
Abbreviated Title:	High-Risk Skin Cancers with Atezolizumab plus NT-I7
Study Phase:	Phase 1b/2a
IND Sponsor:	NeoImmuneTech, Inc. 2400 Research Blvd, Suite 250 Rockville, MD 20850 info@neoimmunetech.com www.neoimmunetech.com
Coordinating Center:	Immune Oncology Network (ION), Fred Hutchinson Cancer Center
Principal Investigator:	[redacted]
Participating Organizations:	ION – Immune Oncology Network member sites

ION Protocol Manager: [redacted]

Statistician: Cytel, Inc.
Waltham, MA 02451
[redacted]

IND #: 140311

CONTACT INFORMATION	
For regulatory requirements:	**For patient enrollment and data submission:**
Regulatory documentation must be submitted to IQVIA via email to NIT106EDP@iqvia.com	Patient Enrollment and Data collection for this study will be done exclusively through an electronic data capture system. Please see the data submission section of the protocol for further instructions.
For all study-related questions:	
Contact the ION Coordinating Center [redacted]	

SPONSOR SIGNATORY

I have read this protocol in its entirety and agree to conduct the study accordingly:

PROTOCOL AMENDMENT SUMMARY OF CHANGES TABLE

Table 1 – Document History

Document	Date	Substantial	Region
Original Protocol (v1.0)	06Dec2018	-	-
Version 2.0	02Jan2019	FDA Information Request	
Version 3.0	27Feb2019	FDA recommendations and Roche safety notification	
Amendment 1, Version 1.0	09Sep2019	- Protocol revisions per Atezolizumab IB v14, October 2018 - Immunogenicity testing for Atezolizumab no longer required - Administrative corrections	
Amendment 2, Version 1.0	20Apr2020	- Phase 1b Dose Level changes per new available data - Protocol revisions per Atezolizumab IB v15, July 2019 and Atezolizumab IB v15 Addendum 2, December 2019 - Protocol revisions per NT-I7 IB v5, January 29, 2020 - Administrative corrections	
Amendment 3, Version 1.0	23Apr2021	- Protocol revisions per Atezolizumab IB v17, September 2020 - Protocol revisions per NT-I7 IB v6, February 19, 2021 - Added 2 timepoints for Serum Chemistry in Study Calendar Table - Corrected Chemical formula and Structural formula for NT-I7 - NT-I7 dosing schedule changed from Q3W to Q6W (every other cycle) per new available data - Pharmacokinetic draw schedule modified per new available data - Administrative corrections	
Amendment 4, Version 1.0	11MAR2022	- Protocol revisions per Atezolizumab IB v18, July 2021 and Atezolizumab IB v18 Addendum 1, August 2021 - Changed irRC to iRECIST - Clarification to Exclusion Criteria - Added notes, clarification to current protocol language - Administrative corrections	
Amendment 5, Version 1.0	19JAN2023	- Updated RP2D to 1200 μg/kg	

		- Protocol revisions per NT-I7 IB v8.0, 19SEP2022 - Protocol revisions per Atezolizumab IB v18, July 2021 and Addendum 1, August 2021 - Protocol revisions per Dear Investigator Letters, dated July 27, 2022 and November 21, 2022 - Administrative corrections and additions	

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1. PROTOCOL SUMMARY

1.1 Synopsis

Abbreviated Title	High Risk Skin Cancers with Atezolizumab plus NT-I7
Trial Phase	Phase 1b/2a
Clinical Indication	Anti-programmed cell death protein 1 (PD-1)/ programmed death-ligand 1 (PD-L1) naïve or relapsed/refractory high-risk skin cancers: melanoma, Merkel cell carcinoma (MCC) and cutaneous squamous cell carcinoma (cSCC)
Trial Type	Phase 1b/2a, non-randomized 2 arm, open label trial with initial NT-I7 dose escalation phase (phase 1b) followed by a dose expansion phase (phase 2a)
Primary Objective	To evaluate the safety and tolerability of NT-I7 in combination with atezolizumab (Phase 1b), including estimation of the maximum tolerated dose (MTD) and/or the recommended Phase 2 dose (RP2D) Phase 2a: To evaluate the Objective Response Rate (ORR) according to RECIST v1.1 and iRECIST, as determined by the investigator
Secondary Objectives	To evaluate immunogenicity of NT-I7 in combination with atezolizumab To make a preliminary assessment of the anti-tumor activity of NT-I7 in combination with atezolizumab
Exploratory Objectives	To make a preliminary assessment of PK parameters of NT-I7 in combination with atezolizumab To make a preliminary assessment of biomarkers that might act as pharmacodynamic indicators of activity of NT-I7 in combination with atezolizumab To make a preliminary assessment of biomarkers that might act as predictors of anti-tumor activity of NT-I7 in combination with atezolizumab
Dose Escalation Phase (Phase 1b)	NT-I7 will be escalated until a plateau of peripheral T-cell level and tumor infiltration of T-cells is reached or unacceptable toxicities.
End Points	Primary endpoint of safety and tolerability of NT-I7 in combination with atezolizumab and estimation of MTD or RP2D for the combination. Secondary endpoints of immunogenicity of NT-I7, Objective Response Rate (ORR), Disease Control Rate (DCR), Duration of Objective Response (DOR), Progression-Free Survival (PFS) and Overall Survival (OS).
Arm I (Phase 2a)	Anti-PD-1/PD-L1 naïve cSCC and MCC
Arm II (Phase 2a)	Anti-PD-1/PD-L1 relapsed/refractory MCC, cSCC and melanoma

Number of Trial Patients	Approximately 84 patients
Number of Clinical Sites	Approximately 8 clinical research sites are expected to participate in this study
Duration of Participation	Each patient will participate in the trial from the time the Informed Consent Form (ICF) is signed through final protocol-specified contact. The active study will end when the last patient completes the 90-day safety follow up visit, approximately 27 months after enrollment.
Estimated Duration of Trial	Trial is expected to be completed approximately 36 months after the last patient is enrolled.

1.2 Schema

Description of Study

This is a Phase 1b/2a, open-label, multicenter study to evaluate the safety, tolerability and anti-tumor effect of NT-I7 (recombinant human interleukin 7 [rhIL]-7-hybrid Fc [hyFc]) in combination with atezolizumab (MPDL3280A) in patients with anti-PD-1/PD-L1 naïve or relapsed/refractory high-risk skin cancers including cSCC, MCC and melanoma.

This study has been designed to allow for an investigation of MTD or RP2D of NT-I7 in combination with atezolizumab. There are two phases to this study:

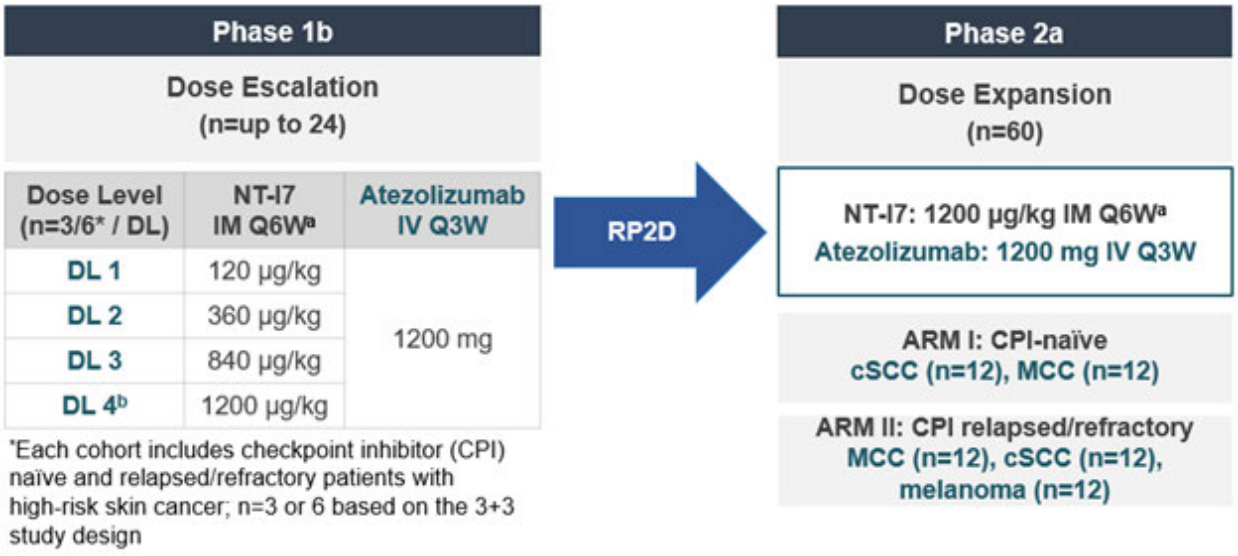
- Phase 1b, a NT-I7 dose-escalation phase to determine the MTD or RP2D
- Phase 2a, a non-randomized parallel dose expansion phase to confirm the MTD or RP2D in both arms.

Arm I: Anti-PD-1/PD-L1 (checkpoint inhibitors [CPI]) naïve patients with cSCC and MCC

Arm II: Anti-PD-1/PD-L1 relapsed/refractory patients with MCC, cSCC and melanoma

Number of Patients

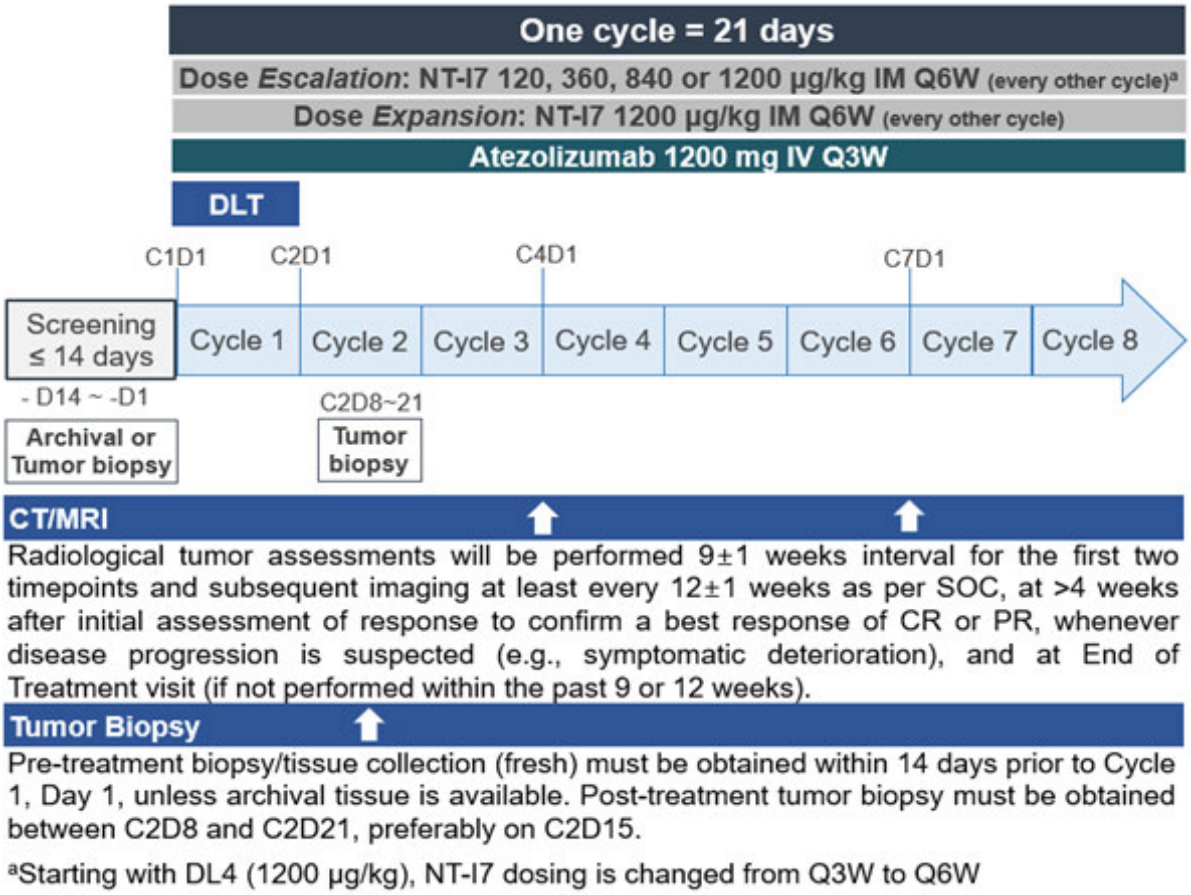
A total of approximately 84 patients will be enrolled; up to approximately 24 patients will be enrolled in the Phase 1b (3 + 3 dose escalation design will be used), and approximately 60 patients will be enrolled in the Phase 2a (24 patients in Arm I, i.e., 12 patients for each indication, and 36 patients in Arm II, i.e., 12 patients for each indication).



[a]NT-I7 Dose Interruption

NT-I7 administration will be skipped when the ALC is higher than 10,000 cells/µL on Day 1 of every other cycle, starting on Cycle 3. Blood draws conducted prior to dosing on Day 1 will determine if NT-I7 should be interrupted for the current cycle.

[b]Starting with DL4 (1200 µg/kg), NT-I7 dosing is changed from Q3W to Q6W. Atezolizumab dosing remains the same (Q3W).



2. INTRODUCTION

2.1 Study Rationale

Very few preclinical studies have combined interleukin 7 (IL-7) with programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), or CTLA-4 checkpoint blockade. One murine study showed the therapeutic efficacy of concomitant blockade of CTLA-4 and PD-1 relies on interdependence of IL-7 and interferon-gamma (IFN-γ) signaling in T-cells, predicting the exogenous IL-7 will boost the therapeutic efficacy 1. Another study of murine sepsis showed that IL-7 reverses immune suppression by increasing lymphocyte proliferation, expression of lymphocyte adhesion molecules, IFN-γ production, and CD28 expression on splenic CD8+ T-cells. Combined treatment with IL-7 and anti-PD-1 produced additive effects on CD28 expression, lymphocyte proliferation, and splenic secretion of IFN-γ 2. A recent Science paper 3 using the mouse model of chronic lymphocytic choriomeningitis virus (LCMV) infection explored the combined effect of IL-7 and anti-PD-L1 on the development and functionality of exhausted T-cells. Anti-PD-L1 treatment moderately improved IL-7 signaling by increasing CD127 (IL-7Rα) expression on exhausted T-cells and exploited potential pathways to improve checkpoint blockade efficacy by combining with IL-7.

Anti-PD-1 and anti-PD-L1 including atezolizumab can induce remarkable responses in a subset of patients with cancer including skin cancers, yet very few are cured. The mechanisms of

immune escape, including (1) the lack of strong, tumor-specific antigens or epitopes recognized by T-cells, (2) impaired or suppressed antigen presentation machinery including downregulation of major histocompatibility complex on cancer cells, (3) impaired or suppressed cytotoxic T-cell activation, (4) poor infiltration of T-cells into the tumor microenvironment (TME), and (5) increased immunosuppressive cytokines and cells in the TME prevent a large proportion of cancer patients from deriving clinical benefit from anti-PD-1/PD-L1 therapies 4. Thus, additional therapies to increase the frequency and depth of responses to anti-PD-1/anti-PD-L1 such as atezolizumab are needed.

The current trial will determine whether:

1. Administration of NT-I7 at a dose known to expand T-cell number and repertoire will; (a) increase the number of tumor-infiltrating T-cells; and (b) thereby increase the efficacy of atezolizumab therapy.
2. Administration of NT-I7 with atezolizumab will result in clinically meaningful increases in objective response rate (ORR), disease control rate (DCR), duration of objective response (DOR), progression-free survival (PFS) and overall survival (OS).
3. Assessment of serial tumor biopsies to quantify NT-I7 - induced increases in T-cell infiltration will explore the effect of NT-I7 in combination with atezolizumab on TME, e.g., persistent myeloid infiltration, or overexpression of indoleamine 2,3-dioxygenase (IDO), arginase and transforming growth factor β (TGF-β).

2.2 Background - Study Disease(s)

The proposed clinical trial is a phase 1b/2a, open-label, multicenter study of the administration of atezolizumab (MPDL3280A) in combination with NT-I7 in patients with anti-PD-1/PD-L1 naïve or relapsed/refractory high-risk skin cancers: cutaneous Squamous Cell Carcinoma (cSCC), Merkel Cell Carcinoma (MCC) and melanoma.

High risk melanoma, MCC and cSCC share many attributes. Biologically, as a group they have the highest mutational burdens from skin exposure to ultraviolet (UV) radiation and accordingly have amongst the highest response rates to inhibition with PD-1/PD-L1 blockade in the range of 30-40+% ORR. Unfortunately, PD-1/PD-L1 blockade fails to induce complete responses (CRs) in most patients.

Therapeutically, these are surgical diseases before dissemination and are usually treated by the same set of physicians (dermatologists, cutaneous surgical oncologists and cutaneous-based oncologists). Thus, clinical trials with all three diseases utilizing the same group disease and clinical trial experts are practical and can be efficient.

Investigational trials of checkpoint inhibitors in all 3 disease groups have shown similar ORR (see table below).

Tumor Type	Agent/Phase trial	ORR
cSCC	Cemiplimab (Phase II) Two advanced cSCC expansion cohorts (Phase I)	47% https://investor.regeneron.com/news-releases/news-release-details/fda-approves-libtayor-cemiplimab-rwlc-first-and-only-treatment
MCC	Pembrolizumab (Phase II) Avelumab (Phase II)	33%-56% 5 6
Melanoma	Pembrolizumab (Phase III) Nivolumab (III)	33%-40% 7 8

Abbreviations: cSCC=cutaneous squamous cell carcinoma; MCC=Merkel cell carcinoma; ORR=objective response rate.

IL-7 is a homeostatic growth factor for T-cells that normally contributes to maintaining the normal number of peripheral blood T-cells. IL-7-mediated signaling can induce proliferation in the absence of T-cell receptor (TCR) signaling 9. IL-7 signals through the IL-7 receptor (IL-7R) heterodimer composed of the common γ-chain (CD132) cytokine receptor and a unique IL-7Rα (CD127). During normal conditions, IL-7R expression is maintained on resting T-cells 10 and exogenous IL-7 induces expansion of most categories of T-cells.

In clinical studies IL-7 has been shown to have numerous characteristics that could provide synergism with anti-PD-L1:

1. Increase peripheral blood T-cells by several-fold with little toxicity 11-15,
2. Expand all categories of T-cells including CD8+, CD4+ and memory T-cells 12,
3. Increase the total body complement of T-cells including increased size of lymph nodes, spleen, liver 16,
4. Increase TCR diversity by preferentially stimulating the growth of T-cell receptor excision circles (TREC)-positive, naïve T-cells 16. Continuous signaling by IL-7 induces anti-apoptotic and co-stimulatory responses that are essential for the survival of naïve T-cells.
5. Increase tissue infiltration of T-cells into gut mucosa (other tissues including tumor have not been examined) 17.
6. Reverse neutrophil lymphocyte ratios [Cancer Immunotherapy Trials Network (CITN)12-03 (NCT01881867), manuscript submitted]
7. Augment immune-mediated control of both tumors and viruses 10.

Very few preclinical studies have combined IL-7 with PD-1 or PD-L1 checkpoint blockade and there have been no human trials for these combinations, in large part due to a lack of availability of long-acting rhIL-7. However, there is sufficient inferential data to support testing the combination in a clinical trial setting.

The current trial will test whether addition of NT-I7 (rhIL-7- hyFc), a long-acting IL-7, to atezolizumab, using a NT-I7 regimen known to increase the total body complement of T-cells, will increase the frequency and/or depth of clinical responses to atezolizumab monotherapy and, thereby, provide clinically meaningful outcomes for patients with anti-PD-1/PD-L1 naïve or relapsed/refractory high-risk melanoma, MCC and cSCC.

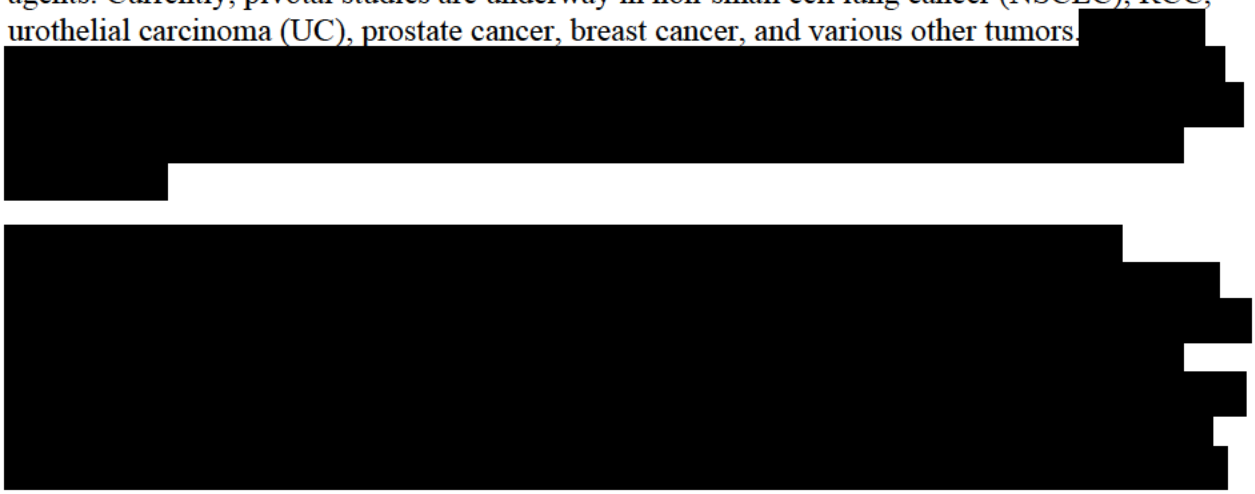
2.3 Agent(s)

2.3.1 Atezolizumab (MPDL3280A)

TECENTRIQ® (atezolizumab) is a humanized immunoglobulin G1 (IgG1) monoclonal antibody that is produced in Chinese hamster ovary (CHO) cells. Atezolizumab targets PD-L1 on tumor-infiltrating immune cells (ICs) or tumor cells (TCs) and prevents interaction with the PD-1 receptor and B7.1 (CD80), both of which function as inhibitory receptors expressed on T-cells and other immune cells. Interference of the PD-L1:PD-1 and PD-L1:B7.1 interactions may enhance the magnitude and quality of the tumor-specific T-cell response through increased T-cell priming, expansion and/or effector function. Atezolizumab was engineered to eliminate Fc-effector function via a single amino acid substitution at position 298 on the heavy chain, which results in a non-glycosylated antibody that has minimal binding to Fc receptors and, consequently, eliminates detectable Fc-effector function. By eliminating Fc-effector function and antibody-dependent T-cell-mediated cytotoxicity, antibody-mediated clearance of activated effector T-cells is also eliminated.

Expression of PD-L1 is prevalent among many human tumors 18, and its overexpression is associated with poor prognosis for patients with certain cancers 19-22. Therefore, interruption of the PD-L1/PD-1 pathway represents an attractive strategy to reinvigorate tumor-specific T-cell immunity.

Treatment with atezolizumab has shown anti-tumor activity in many tumor types, including lung cancer, bladder cancer, melanoma, renal cell carcinoma (RCC), colon cancer, ovarian cancer, gastric cancer, breast cancer, head and neck carcinoma, and hematologic malignancies. Atezolizumab has the potential to produce durable efficacy outcomes. Atezolizumab is being evaluated both as a single agent and in combination with other chemotherapies and targeted agents. Currently, pivotal studies are underway in non-small cell lung cancer (NSCLC), RCC, urothelial carcinoma (UC), prostate cancer, breast cancer, and various other tumors.



Refer to the Atezolizumab IB for additional details on the safety and efficacy of atezolizumab.

2.3.2 NT-I7 (rhIL-7-hyFc)

NT-I7 is being co-developed by NeoImmuneTech, Inc. and its partner Genexine, Inc. for use in the treatment of cancer patients and in conditions where lymphocyte number and/or function is suppressed. NT-I7 is a long-acting IL-7 cytokine consisting of human IL-7 and a hybrid crystallizable fragment (hyFc) region, which extends its half-life. The active ingredient is recombinant human interleukin-7 fused to a hybrid Fc region of a human antibody (rhIL-7-hyFc). HyFc® is composed of the human immunoglobulin D (IgD) hinge region fused to the N-terminal region of homologous constant region domain 2 (CH2) from IgD, which is in turn fused to the C terminal region of CH2 and the entire homologous constant region 3 (CH3) region of the antibody immunoglobulin G4 (IgG4). A detailed structural diagram of NT-I7 is shown in the NT-I7 IB.

In preclinical studies, NT-I7 has demonstrated potent anti-tumor efficacy, as well as an increase in peripheral T-cells and tumor infiltrating lymphocytes as a monotherapy, in combination with chemo/radiotherapy, and in combination with immune checkpoint inhibitors (CPIs).

Recombinant human IL-7 (rhIL-7), previously developed by Cytheris, was administered to over 390 subjects in clinical trials, including patients with chronic human immunodeficiency virus (HIV) or hepatitis C virus (HCV) infections, patients with refractory solid tumors, and patients after allogeneic stem cell transplantation 23. These combined clinical studies demonstrated proof

of mechanism for the use of exogeneous IL-7 in patients with regard to substantial increases in T-cell count, tolerability and overall safety profile. Cytheris' rhIL-7 therapeutic platform faced fundamental technical challenges, however, with a short half-life, lack of molecular stability due to an intrinsically unstable protein and poor production yield. NT-I7 overcomes the fundamental problems that stalled previous rhIL-7 programs by fusing the C-terminal of IL-7 to its proprietary hyFc long-acting platform.

[redacted]

Refer to the NT-I7 IB for additional details on the safety of NT-I7.

2.4 Overall Risk/Benefit and Ethical Assessment

T-cells play a pivotal role in inducing antigen-specific immune responses to attack cancer cells, as they can recognize cancer antigens, destroy cancer cells and differentiate into memory T-cells to facilitate long term immunity. The anti-tumor efficacy of T-cells can be enhanced by increasing the diversity of the T-cell receptor repertoire to enable recognition of specific antigens expressed by cancer cells, expanding T-cell clones responsive to tumor specific antigens and accelerating differentiation to memory T-cells to increase tumor tissue infiltration [27,28]. Chemotherapy and radiotherapy are widely used as standard of care for the majority of cancer patients, but these can kill T-cells and compromise overall anti-cancer efficacy by suppressing bone marrow function and destroying immune cells in the blood [29,30].

T-cell lymphopenia in cancer patients is associated with lower clinical anti-tumor responses and lower survival rates. To date, IL-2 (Proleukin® [aldesleukin]) is the only Food and Drug Administration (FDA)-approved cytokine product available as a therapeutic option to induce the proliferation and activation of T-cells. However, the clinical application of IL-2 is very limited due to serious adverse effects such as capillary leak syndrome and compromised efficacy through the increased proliferation of regulatory T-cells that inhibit anti-tumor immune responses [31,32].

IL-7 is a crucial factor for the growth and activation of T-cells, and serves as a key player in the differentiation, proliferation and survival of naïve and memory T-cells. Importantly, it does not induce proliferation of regulatory T-cells. IL-7 is also referred to as a homeostatic cytokine, as IL-7 receptors (CD127) expressed on the surface of T-cells are downregulated or internalized if T-cells are overactivated by IL-7. To date, there have been no reports of rhIL-7 (CYT107 or CYT99007, Cytheris, Inc.) causing cytokine release syndrome, a serious immune-mediated adverse reaction caused by excessive immune activation associated with other cytokine therapeutics [11-16,33-44].

The protocol will administer NT-I7 via the IM route starting from 120 µg/kg, the dose that is only 1/4 of the dose currently being tested in the Phase 1b clinical study with solid tumor patients in Korea (480 µg/kg). The dose will be escalated up to 1200 µg/kg to find the MTD and/or the RP2D.

With no specific overlapping target organs toxicities identified in nonclinical or clinical studies in the individual single-agent toxicology studies for NT-I7 and atezolizumab, evidence suggests a very low potential for overlapping toxicity in patients treated with NT-I7 in combination with atezolizumab.

Taken together, data from non-clinical and clinical studies suggest that the addition of NT-I7 to atezolizumab, using an NT-I7 regimen known to increase the total body complement of T-cells, has the potential to increase the frequency and/or depth of clinical responses to atezolizumab monotherapy and, thereby, provide clinically meaningful outcomes for patients with anti-PD-1/PD-L1 naïve or relapsed/refractory high-risk melanoma, MCC and cSCC.

More detailed information about the known and expected benefits and risks and reasonably expected AEs of atezolizumab and NT-I7 may be found in the respective IBs.

The Sponsor (NeoImmuneTech), via the Immune Oncology Network (ION) Coordinating Center, will immediately notify the Principal Investigators (PIs) if any additional safety or toxicology information becomes available during the study.

This study will be performed in compliance with the protocol, International Council for Harmonisation (ICH) Good Clinical Practice (GCP) and applicable regulatory requirements. Aspects of the study concerned with the investigational medicinal product(s) (IMPs) will meet the requirements of European Union – Good Manufacturing Practice (EU GMP).

3. OBJECTIVES AND ENDPOINTS

This study will evaluate the safety and tolerability and assess the antitumor efficacy of NT-I7 in combination with atezolizumab (Phase 1b/2a) in patients with anti-PD-1/PD-L1 naïve or relapsed/refractory high-risk skin cancers (cSCC, MCC, melanoma). Specific objectives and corresponding endpoints for the study are outlined in Table 2.

Table 2 – Study Objectives and Endpoints

Objectives	Corresponding Endpoints
Primary Objective	
To evaluate the safety and tolerability of NT-I7 in combination with atezolizumab (Phase 1b), including estimation of the Maximum Tolerated Dose (MTD) and/or the Recommended Phase 2 Dose (RP2D)	- Incidence, nature, and severity of adverse events graded according to NCI CTCAE v5.0 - Incidence and nature of DLTs - Potential correlation with PK, pharmacodynamic, safety, and efficacy parameters
Phase 2a: To evaluate the Objective Response Rate (ORR) according to RECIST v1.1 and iRECIST, as determined by the investigator	- Objective Response Rate (ORR), defined as the percentage of patients who have at least one confirmed partial response (PR) or complete response (CR) according to RECIST v1.1 and iRECIST, as determined by the investigator
Secondary Objectives	
To evaluate immunogenicity of NT-I7 in combination with atezolizumab	- Incidence of anti-drug antibody (ADA) to NT-I7 during the study relative to the prevalence of ADA at baseline
To make a preliminary assessment of the anti-tumor activity of NT-I7 in combination with atezolizumab	- Disease Control Rate (DCR), defined as proportion of patients with a best overall response of CR, PR or stable disease (SD). - Duration of objective response (DOR), defined as the time from the first occurrence of a documented objective response to the time of the first documented disease progression or death from any cause, whichever occurs first, per RECIST v1.1 and iRECIST as determined by the investigator - Progression Free Survival (PFS), defined as the time from the first study treatment (Cycle 1, Day 1) to the first occurrence of progression or death from any cause, whichever occurs first, per RECIST v1.1 and iRECIST as determined by the investigator - Overall survival (OS), defined as the time from first study treatment (Cycle 1, Day 1) to death from any cause
Exploratory Objectives	
To make a preliminary assessment of PK parameters of NT-I7 in combination with atezolizumab	The endpoints will include the evaluation of the effect of the investigational treatment combination on the tumor microenvironment, based upon

Objectives	Corresponding Endpoints
To make a preliminary assessment of biomarkers that might act as pharmacodynamic indicators of activity of NT-I7 in combination with atezolizumab To make a preliminary assessment of biomarkers that might act as predictors of anti-tumor activity of NT-I7 in combination with atezolizumab	baseline and post-baseline tumor biopsy comparisons of: - Serum concentration of NT-I7 administered in combination with atezolizumab at specified timepoints for the following parameters: Area under the concentration time-curve (AUC) Maximum serum concentration (Cmax) Minimum serum concentration (Cmin) Clearance (CL) - Number, distribution, and phenotype of tumor-infiltrating cells - PD-L1 expression - Expression of Interferon γ (IFNγ) and associated inflammatory gene expression in the tumor microenvironment - Changes in tumor microenvironment that correlate with response or provide information on potential actionable causes for lack of clinical benefit

Abbreviations: ADA=anti-drug antibodies; AUC=area under the curve; CL=clearance; C_{max}=maximum serum concentration; C_{min}=minimum serum concentration; CR=complete response; CTCAE=Common Terminology Criteria for Adverse Events; DCR=disease control rate; DLT=dose limiting toxicity; DOR=duration of objective response; IFN-γ=interferon-gamma; iRECIST=immune-related Response Evaluation Criteria in Solid Tumors; MTD=maximum tolerated dose; NCI=National Cancer Institute; ORR=objective response rate; OS=overall survival; PFS=progression-free survival; PK=pharmacokinetic; PR=partial response; RECIST=Response Evaluation Criteria in Solid Tumors; RP2D=recommended Phase 2 dose; SD=stable disease.

4. PATIENT SELECTION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

4.1 Inclusion Criteria

Patients must meet all the following criteria for study entry:

General Inclusion Criteria

1. Patients must have the ability to understand and the willingness to sign a written informed consent document.

2. Patients must be ≥18 years of age on day of signing informed consent document.

   Because no dosing or AE data are currently available on the use of NT-I7 in patients <18 years of age, children are excluded from this study.

3. Eastern Cooperative Oncology Group (ECOG) performance status ≤2 (Karnofsky ≥60%, see Appendix A). Exceptions, such as bilateral amputation for trauma, could be approved following consultation with the Protocol PI and/or ION Coordinating Center.

4. Patients must have a life expectancy of greater or equal to 12 weeks.

5. Patients must have adequate organ and marrow function as defined below:

– Absolute neutrophil count	≥1,000/mcL
– Platelets	≥100,000/mcL
– Hemoglobin	≥8.0 g/dL
– Total bilirubin	≤1.5 × institutional upper limit of normal (ULN) (however, patients with known Gilbert's disease who have serum bilirubin level ≤3 × ULN may be enrolled.)
– AST(SGOT)/ALT(SGPT)	≤3 × ULN (AST and/or ALT ≤5 × ULN for patients with liver involvement.)
– Alkaline phosphatase	≤2.5 × ULN (≤5 x ULN for patients with documented liver involvement or bone metastases.)
– Creatinine clearance	≥30 mL/min/1.73 m^2 by Cockcroft-Gault: $\frac{(140 - \text{age}) \times (\text{weight in kg}) \times (0.85 \text{ if female})}{72 \times (\text{serum creatinine in mg/dL})}$ At the discretion of the treating physician, a 24-hour urine creatinine clearance could be obtained and utilized as the gold standard if creatinine clearance by Cockcroft-Gault is <30, and prevents patient enrollment on the trial.
– INR and aPTT	≤1.5 × ULN (This applies only to patients who do not receive therapeutic anticoagulation; patients receiving therapeutic anticoagulation, such as low-molecular-weight heparin or warfarin, should be on a stable dose.)

6. Patients positive for HIV are allowed on study, but HIV-positive patients must have:

   a. A stable regimen of highly active antiretroviral therapy (HAART)

   b. No requirement for concurrent antibiotics or antifungal agents for the prevention of opportunistic infections

   c. A CD4+ count above 100 cells/mcL and an undetectable HIV viral load on standard polymerase chain reaction (PCR)-based tests

7. Female patients of childbearing potential (including women who have had a tubal ligation) must have a negative serum pregnancy test within 72 hours prior to Cycle 1, Day 1 and must agree to remain abstinent (refrain from heterosexual intercourse) or use contraceptive methods that result in a failure rate of < 1% per year during the study treatment period and for at least 150 days after the last dose of study agent(s) – refer to Section 5.5 for further details.

8. Men must agree to remain abstinent (refrain from heterosexual intercourse) or use contraceptive methods that result in a failure rate of < 1% per year during the study treatment period and for at least 150 days after the last dose of study agent(s). Men must agree to refrain from donating sperm during this same period – refer to Section 5.5 for further details.

Cancer-Specific Inclusion Criteria

9. Patients must have histologically or cytological documented locally advanced/inoperable or metastatic skin cancer including melanoma, MCC and cSCC.

   Note: Cancer must be invasive; carcinoma in situ is excluded.

   Note: Patients must have cutaneous melanoma; mucosal melanoma is excluded.

10. **Arm I**: Anti-PD-1/PD-L1 naïve cSCC and MCC.

    Arm II: Anti-PD-1/PD-L1 relapsed/refractory MCC, cSCC and melanoma.

    Note: Each arm and each cancer type have different eligibility criteria (see below).

    Arm I – cSCC: Patients must have biopsy-proven metastatic cSCC or locoregional cSCC that has recurred following standard locoregional therapy with surgery and/or radiation therapy.

    Arm I – MCC: Patients must have biopsy-proven metastatic MCC or locoregional MCC in need of systemic therapy, including patients that have not had prior systemic therapy or have recurred following standard locoregional therapy with surgery and/or radiation therapy. Prior chemotherapy is allowed.

    Note: For Arm II patients, eligibility will be determined by clinical progression or radiographic progression. Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 will be used for patients on prior protocols that have mandated serial RECIST readings. In addition, the following criteria will be applied for each tumor types.

    Arm II – MCC: Patients must have biopsy-proven metastatic MCC or locoregional MCC that has recurred following anti-PD-1 or anti-PD-L1, or has stable disease (SD) following anti-PD-1 or anti-PD-L1, defined as a minimum of 6 weeks of SD per RECIST 1.1 criteria. Patients may have also failed other systemic regimens, e.g., chemotherapy.

Note: Progression following investigational therapies is allowed.

Arm II – cSCC: Patients must have biopsy-proven metastatic cSCC or locoregional cSCC that has recurred following anti-PD-1 or anti-PD-L1, or has SD following anti-PD-1 or anti-PD-L1, defined as a minimum of 6 weeks of SD per RECIST 1.1 criteria.
Note: Progression following investigational therapies is allowed.

Arm II – melanoma: Patients must have biopsy-proven metastatic melanoma or locoregional melanoma that has recurred following anti-PD-1, anti-PD-L1, or has SD following anti-PD-1 or anti-PD-L1, defined as a minimum of 6 weeks of SD per RECIST 1.1 criteria.

Note: Prior therapy with ipilimumab is allowed (subject to a 6-week washout period) but not required.

Note: Progression following targeted therapies (e.g., BRAF inhibitor and/or MEK inhibitor) or other approved (e.g., talimogene laherparepvec [T-VEC]) or investigational therapies is allowed.

11. Patients must have measurable disease per clinical exam or per RECIST 1.1 assessed by computed tomography (CT) scan or magnetic resonance imaging (MRI). See Section 11 for the evaluation of measurable disease.

12. Patients must have biopsiable disease (i.e., have at least 1 tumor lesion that is accessible and feasible to biopsy) as determined by the treating physician.

13. Patients must provide tissue from an archival tumor sample (obtained after last therapy) or newly obtained (preferred) core biopsy, punch biopsy, or surgical excision of a primary or metastatic tumor lesion.

4.2 Exclusion Criteria

Patients who meet any of the following criteria will be excluded from study entry.

General Exclusion Criteria

1. Inability to comply with study and follow-up procedures.

2. Pregnancy, lactation, or breastfeeding.

3. Significant cardiovascular disease, such as New York Heart Association cardiac disease (Class II or greater), myocardial infarction (within the last 3 months), uncontrolled hypertension, unstable arrhythmia and/or unstable angina.

4. Poorly controlled Type 2 diabetes mellitus defined as a screening hemoglobin A1C ≥8% or a fasting plasma glucose ≥160 mg/dL (or 8.8 mmol/L).

5. Major surgical procedure, other than for diagnosis, within 28 days prior to Cycle 1, Day 1, or anticipation of need for a major surgical procedure during the study.

6. Any other diseases, metabolic dysfunction, physical examination finding and/or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of an investigational drug or that may affect the interpretation of the results or may render the patient at high risk from treatment complications.

7. Prisoners or subjects who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or medical (e.g., infectious) illness.

Cancer-Specific Exclusion Criteria

8. Patients who have had chemotherapy or radiotherapy within 2 weeks (4 weeks for nitrosoureas or systemic mitomycin C) prior to Cycle 1, Day 1.

9. Patients who had prior treatment with immune CPIs, immunomodulatory monoclonal antibodies (mAbs), and/or mAb-derived therapies within 6 weeks before the initiation of study treatment, except for:

    - Prior anti-PD-L1/anti-PD-1 requires a 3-week washout period.
    - Prior anti-CTLA-4 requires a 6-week washout period.

10. Patients who have not recovered from AEs (other than alopecia, vitiligo, neuropathy or endocrinopathy managed with replacement therapy) due to agents administered more than 4 weeks earlier (i.e., have residual toxicities >Grade 1). However, the following therapies are allowed:
    - Hormone-replacement therapy or oral contraceptives
    - Herbal therapy ≥1 week before initiation of study treatment (herbal therapy intended as anticancer therapy must be discontinued at least 2 weeks before initiation of study treatment)
    - Palliative radiotherapy for bone metastases >2 weeks prior to Cycle 1, Day 1.

11. Patients who have received treatment with any other investigational agent within 4 weeks prior to Cycle 1, Day 1.

12. Patients who have received treatment and failed therapy with checkpoint inhibition plus a T-cell growth factor, e.g., IL-2 (NTKR-204), IL-15 (ALT-803) or IL-7 (CYT107).

13. Patients who have a history of an immune-related Grade 3 or 4 AE attributed to prior cancer immunotherapy (other than endocrinopathy managed with replacement therapy or asymptomatic elevation of serum amylase or lipase) that resulted in permanent discontinuation of the prior immunotherapeutic agent and/or occurred within 6 months prior to Cycle 1, Day 1.

14. Patients who have not completely recovered from irAEs (i.e., have residual toxicities >Grade 1) related to prior cancer immunotherapy (other than endocrinopathy managed

with replacement therapy or stable vitiligo). Patients treated with corticosteroids for irAEs must demonstrate absence of related signs or symptoms for ≥ 4 weeks following discontinuation of corticosteroids.

Note: Patients with a history of severe hypersensitivity reactions to prior immunotherapy are not allowed.

15. Patients with known primary central nervous system (CNS) malignancy, untreated CNS metastases, or active CNS metastases (progressing or requiring corticosteroids for symptomatic control) are excluded, with the following exceptions:

    - Patients with a history of treated CNS metastases may be enrolled, provided all the criteria listed above are met as well as the following:
        - Measurable disease outside the CNS
        - No ongoing requirement for corticosteroids as therapy for CNS metastases, with corticosteroids discontinued for ≥ 2 weeks prior to initiation of study treatment
        - Anticonvulsants at a stable dose are allowed
        - Radiographic demonstration of improvement upon the completion of CNS-directed therapy and no evidence of interim progression between the completion of CNS-directed therapy and the screening radiographic study
        - Screening CNS radiographic study ≥4 weeks from completion of radiotherapy.

16. Patients who have leptomeningeal disease.

17. Patients who have uncontrolled tumor-related pain:

    - Symptomatic lesions amenable to palliative radiotherapy (e.g., bone metastases or metastases causing nerve impingement) should be treated prior to enrollment
    - Asymptomatic metastatic lesions whose further growth would likely cause functional deficits or intractable pain (e.g., epidural metastasis that is not currently associated with spinal cord compression) should be considered for loco-regional therapy if appropriate prior to enrollment.

18. Patients who have uncontrolled hypercalcemia (>1.5 mmol/L ionized calcium or calcium >12 mg/dL or corrected serum calcium ≥ ULN) or symptomatic hypercalcemia requiring continued use of bisphosphonate therapy or denosumab.

    Note: Use of bisphosphonate therapy or denosumab specifically to prevent skeletal events and patients who do not have a history of clinically significant hypercalcemia are allowed.

    Note: Patients on denosumab must be willing and eligible to receive a bisphosphonate instead of denosumab while on study.

19. Patients who have spinal cord compression not definitively treated with surgery and/or radiation or previously diagnosed and treated spinal cord compression without evidence that disease has been clinically stable for ≥2 weeks prior to screening.

Treatment-Specific Exclusion Criteria

20. Patients in Arm I (CPI treatment naïve) with autoimmune disease history, including but not limited to systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, vascular thrombosis associated with antiphospholipid syndrome, Wegener's granulomatosis, Sjögren's syndrome, Bell's palsy, Guillain-Barré syndrome, multiple sclerosis, vasculitis or glomerulonephritis.

21. Patients in Arm II (CPI refractory/relapsed) with autoimmune disease history, including but not limited to systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, vascular thrombosis associated with antiphospholipid syndrome, Wegener's granulomatosis, Sjögren's syndrome, Bell's palsy, Guillain-Barre syndrome, multiple sclerosis, vasculitis or glomerulonephritis, with the following caveats:

    - Patients with a history of autoimmune hypothyroidism on a stable dose of thyroid-replacement hormone may be eligible.
    - Patients with controlled Type 1 diabetes mellitus on a stable insulin regimen may be eligible.
    - Patients with eczema, psoriasis, lichen simplex chronicus, or vitiligo with dermatologic manifestations only (e.g., no psoriatic arthritis) may be eligible provided all the following conditions are met:
        - Rash must cover less than 10% of body surface area (BSA)
        - Disease is well controlled at baseline and only requiring low potency topical steroids (e.g., hydrocortisone 2.5%, hydrocortisone butyrate 0.1%, flucinolone 0.01%, desonide 0.05%, aclometasone dipropionate 0.05%)
        - No acute exacerbations of underlying condition within the last 12 months (e.g., not requiring psoralen plus ultraviolet A radiation [PUVA], methotrexate, retinoids, biologic agents, oral calcineurin inhibitors; high potency or oral corticosteroids).

22. Patients who have received treatment with systemic immunosuppressive medications (including, but not limited to, prednisone >10 mg/day, cyclophosphamide, azathioprine, methotrexate, thalidomide and antitumor necrosis factor [anti-TNF] agents) within 2 weeks prior to Cycle 1, Day 1.

    - Patients who have received acute, low dose, systemic immunosuppressant medications (e.g., a one-time dose of dexamethasone for nausea) may be enrolled following consultation with the Protocol PI and/or ION Coordinating Center.
    - The use of inhaled corticosteroids (e.g., fluticasone for chronic obstructive pulmonary disease) is allowed.
    - The use of oral mineralocorticoids (e.g., fludrocortisone for patients with orthostatic hypotension) is allowed.

- Physiologic dose of corticosteroids (≤10 mg/day of prednisone or equivalent) for adrenal insufficiency is allowed.

23. Patients who have a history of idiopathic pulmonary fibrosis, pneumonitis (including drug induced), organizing pneumonia (i.e., bronchiolitis obliterans, cryptogenic organizing pneumonia, etc.), or evidence of active pneumonitis on screening chest CT scan. History of radiation pneumonitis in the radiation field (fibrosis) is permitted.

24. Patients with active hepatitis B (defined as having a positive hepatitis B surface antigen [HBsAg] test at screening).

    - Patients with past or resolved hepatitis B infection (defined as having a negative HBsAg test and a positive IgG antibody to hepatitis B core antigen [anti-HBc]) are eligible. Hepatitis B virus (HBV) DNA must be obtained in these patients prior to Cycle 1, Day 1, and must demonstrate no active infection.
    - Patients with active hepatitis C (positive for HCV antibody) are eligible only if PCR is negative for HCV RNA.

25. Patients with active tuberculosis (TB).

26. Patients who have severe infections within 4 weeks prior to Cycle 1, Day 1, including but not limited to hospitalization for complications of infection, bacteremia or severe pneumonia.

27. Patients who have signs or symptoms of recent infection (not meeting the above criteria for severe infections) within 2 weeks before initiation of study treatment, including the following:

    - Patients who have received oral or intravenous (IV) antibiotics within 2 weeks before initiation of study treatment. Patients receiving prophylactic antibiotics (e.g., for prevention of a urinary tract infection or chronic obstructive pulmonary disease) are eligible.

28. Patients with prior allogeneic bone marrow transplantation or prior solid organ transplantation. Specific exceptions, such as past kidney transplant now on dialysis, could be approved following consultation with the Protocol PI and/or ION Coordinating Center.

29. Patients who have a history of severe allergic, anaphylactic or other hypersensitivity reactions to chimeric or humanized antibodies or fusion proteins.

30. Patients who have received a live, attenuated vaccine within 4 weeks prior to Cycle 1, Day 1 or anticipation that such a live attenuated vaccine be required during the study. Influenza vaccination should be given during influenza season only.

4.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

5. STUDY DESIGN

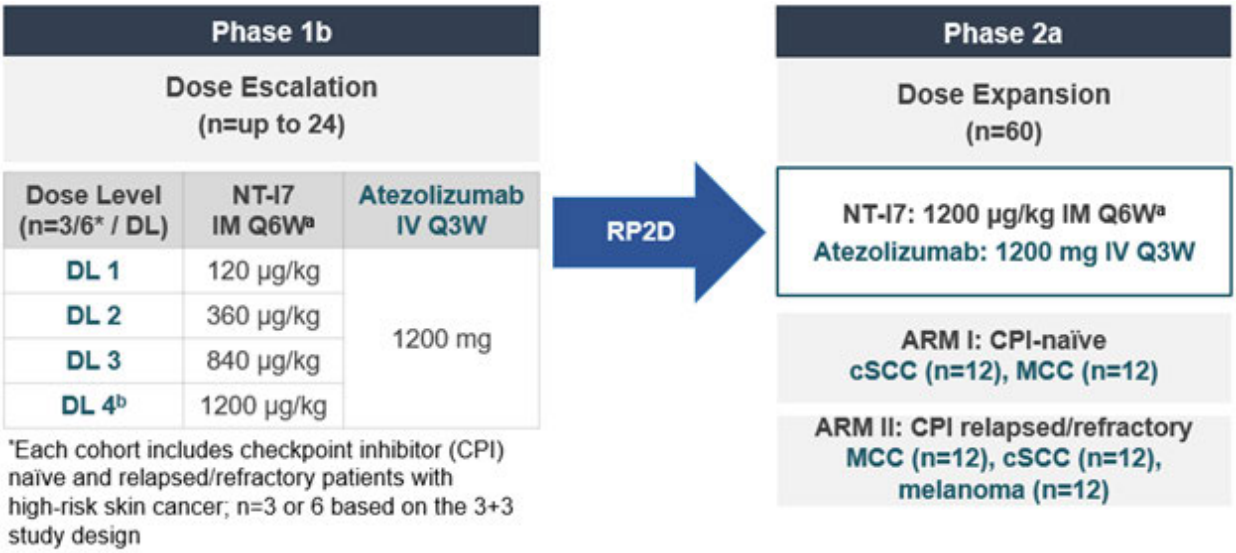
5.1 Overall Design

This is a Phase 1b/2a, non-randomized 2 arm, open label multi-institutional trial. The dose escalation phase (Phase 1b) of the study will determine the MTD or RP2D of NT-I7 in combination with atezolizumab. The Phase 2a will be a non-randomized parallel dose expansion phase to confirm the MTD or RP2D in both arms.

A total of approximately 84 patients will be enrolled; up to approximately 24 patients will be enrolled in the Phase 1b (a 3+3 dose escalation design will be used), and approximately 60 patients will be enrolled in the Phase 2a (24 patients in Arm I, i.e., 12 patients for each indication, and 36 patients in Arm II, i.e., 12 patients for each indication).

Arm I: Anti-PD-1/PD-L1 (CPI) naïve patients with cSCC and MCC

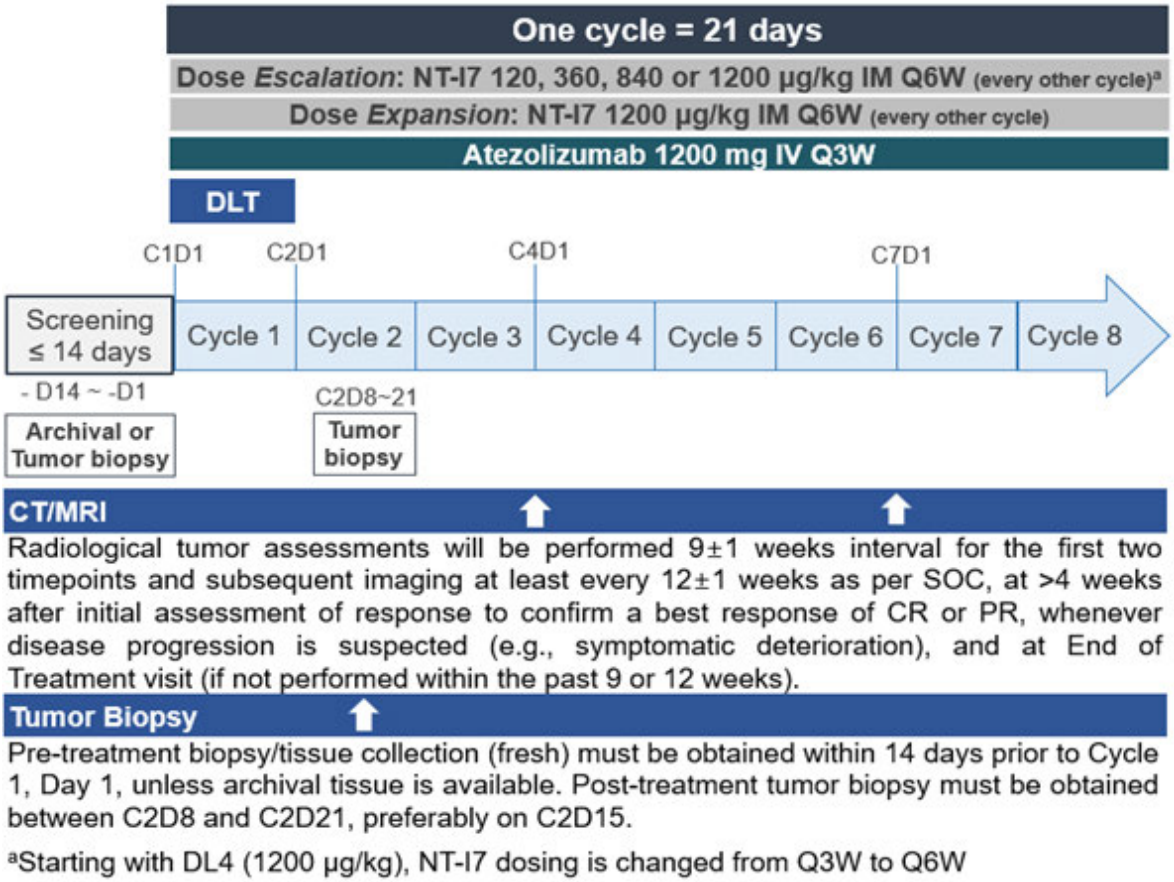
Arm II: Anti-PD-1/PD-L1 relapsed/refractory patients with MCC, cSCC and melanoma



[a]NT-I7 Dose Interruption

NT-I7 administration will be skipped when the ALC is higher than 10,000 cells/µL on Day 1 of every other cycle, starting on Cycle 3. Blood draws conducted prior to dosing on Day 1 will determine if NT-I7 should be interrupted for the current cycle.

[b]Starting with DL4 (1200 µg/kg), NT-I7 dosing is changed from Q3W to Q6W. Atezolizumab dosing remains the same (Q3W).



5.2 Dose Escalation (*Phase 1b*)

A 3 + 3 dose escalation design will be used to assess safety and effect on T-cell level in peripheral blood and tumor. The dose of NT-I7 will be escalated as shown in Section 5.2.2 below.

5.2.1 *Treatment Plan*

Patients will have a required *pre-treatment biopsy* (see Section 5.4) to assess tumor micro-environment and level of T-cell infiltration. Therapy can be started before results of the biopsy are reported, as it will not be used to determine therapy.

Based on the pharmacodynamic data obtained from the GX-I7-CA-003 study (Q3W dosing) and preliminary data from other ongoing studies with less frequent dosing schedules, NT-I7 can be given less frequently than Q3W (e.g., Q6W) and will still lead to similar changes in the ALC and other peripheral blood T-cell subsets.

Starting with Dose Level 4 (1200 µg/kg) of the dose escalation phase, NT-I7 will be administered in combination with atezolizumab every 6 weeks (Q6W). The NT-I7 dose will be given at the same dose level administered in Cycle 1.

On Day 1 of ***every other*** cycle (i.e., Cycle 1, Cycle 3, Cycle 5, etc.), patients will be given a dose of NT-I7 by IM injection before receiving standard atezolizumab (1200 mg, intravenously).

On Day 1 of ***every*** cycle (21-day cycle), patients will be given a dose of Atezolizumab (1200 mg, intravenously). Atezolizumab will be administered every 3 weeks (Q3W).

PK samples will be collected on all patients during the dose escalation phase of the study (refer to PK timepoints tables for specific draw times, Section 12.3.2).

Complete blood count (CBC) with differential including Absolute Lymphocyte Count (ALC) will be checked at each cycle as standard of care. Research blood samples for T-cell phenotypes, CD4+ and CD8+ cell counts will be collected and tested at selected time points (see Sections 12.1.1 and 10).

Starting on Cycle 3, Day 1, NT-I7 administration will be skipped when the ALC is higher than 10,000 cells/µL. When automated and manual ALC counts are performed, manual ALC counts will be used if the results are conflicting. A blood draw will be performed prior to dosing on Day 1. The results will determine if NT-I7 should be interrupted for the current cycle. Treatment with atezolizumab will continue every 3 weeks.

Treatment with both study agents may continue for *up to 2 years* (see Section 6.4).

Patients will have a required *post-treatment biopsy* (see Section 5.4) to assess changes in the tumor micro-environment and the level of T-cell infiltration.

5.2.2 *Dose Escalation*

The starting dose of NT-I7 will be 120 µg/kg. A cohort of 3 patients will be treated at 120 µg/kg and if after the first full cycle (21 days) there are no DLTs, the following dose escalation scheme will begin according to the 3+3 design below.

NT-I7 doses will be escalated according to the following dose escalation scheme,1200 µg/kg as the maximum administered dose (MAD).

Dose Escalation scheme for NT-I7

Dose Level	Dose of NT-I7	Route of Administration
Dose Level 1	120 µg/kg	IM
Dose Level 2	360 µg/kg	IM
Dose Level 3	840 µg/kg	IM
Dose Level 4	1200 µg/kg	IM

Abbreviation: IM=intramuscular.

A 3 + 3 dose escalation design will be used for identifying the MTD and/or the RP2D.

A cohort will initially enroll 3 patients.

- If none (0) of the initial 3 patients in a cohort experiences a DLT, then a new cohort of 3 patients will be treated at the next higher dose level.
- If 1 of the initial 3 patients in a cohort experiences a DLT, then an additional 3 patients will be enrolled at the same dose level.
  - If 0 of these 3 additional patients experiences a DLT (totaling 1/6), a new cohort of 3 patients will be treated at the next higher dose level.
  - If ≥1 of these 3 additional patients experience a DLT (totaling ≥2/6), the dose escalation will stop, and the next lower dose level will be declared the MTD. In the case this happens at the 120 µg/kg dose, a lower dose of 60 µg/kg will be tested.
- If ≥2 of the initial 3 patients in a cohort experience a DLT, then the dose escalation will stop, and the next lower level will be declared the MTD. Three (3) additional patients will be entered at the next lower level if only 3 patients were treated previously at that dose. In the case this happens at the 120 µg/kg dose, a lower dose of 60 µg/kg will be tested following the same 3+3 design (see Appendix C for NT-I7 dilution requirements at specific dose levels).

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enroll 3 patients at the next dose level.
1 out of 3	Enroll at least 3 more patients at this dose level. • If 0 of these 3 additional patients experience a DLT (totaling 1/6), escalate to the next dose level. • If ≥1 of these 3 additional patients experiences DLT (totaling ≥2/6 at the dose level), the dose escalation is stopped, and the next lower dose level will be declared the MTD. In the case this happens at the 120 µg/kg dose level, a lower dose of 60 µg/kg will be tested, and the same 3+ 3 design followed.
≥2 out of 3	Dose escalation will be stopped. The next lower dose level will be declared the MTD. Three (3) additional patients will be entered at the next lower dose level if only 3 patients were treated previously at that dose. In the case this happens at the 120 µg/kg dose level, a lower dose of 60 µg/kg will be tested, and the same 3+3 design followed.

Abbreviations: DLT=dose-limiting toxicity; MTD=maximum tolerated dose.

The DLT is defined in Section 6.2. The DLT evaluation period is the first 21 days (3 weeks) of study treatment (Cycle 1, Day 1 to 21).

The dose escalation decisions will be communicated to the clinical research sites by regularly scheduled teleconferences and follow up email correspondence documenting the decision and rationale.

The RP2D will be selected according to the following logic, taking into account the MTD determination from the Dose Escalation Phase (Phase 1b) and the Maximum Effective Dose (MED) level which is defined as the dose level at which maximum effects on peripheral blood T-cell levels and intratumor T-cell levels are observed. The intratumor T-cell levels will dominate if the peripheral blood and intratumor T-cell levels differ. The available data will be assessed by the Data and Safety Monitoring Committee, which includes the ION Coordinating Center, the Protocol PI, clinical sites PIs and NeoImmuneTech's Chief Medical Officer or designee to select the RP2D.

- If the MTD is determined AND
  - MTD = MED, then the RP2D = MTD = MED
  - MTD > MED, then the RP2D = MED
- If the MTD is not reached, then the RP2D = MED

Once the RP2D has been selected, the trial will proceed to the Dose Expansion Phase (Phase 2a) to further evaluate RP2D in a larger number of patients.

5.3 Expansion Phase (*Phase 2a*)

Patients enrolled into the trial during the Expansion Phase *(Phase 2a)* will be treated at the RP2D level, determined in Phase I to be 1200 µg/kg IM Q6W, as defined in Section 5.2.2.

The Phase 2a is designed to evaluate the anti-tumor effects of NT-I7 in combination with atezolizumab in a total of 60 patients. Twenty-four patients in Arm I, i.e., 12 patients for each indication (cSCC and MCC), and 36 patients in Arm II, i.e., 12 patients for each indication (MCC, cSCC and melanoma), will be enrolled. Enrolled patients will be separated into two arms depending on previous use of anti-PD-1/anti-PD-L1 therapy and cancer type.

Arm I (n=24): Anti-PD-1/PD-L1 naïve patients with cSCC (n=12) and MCC (n=12)

Arm II (n=36): Anti-PD-1/PD-L1 relapsed/refractory patients with MCC (n=12), cSCC (n=12) and melanoma (n=12)

5.3.1 *Treatment Plan*

Patients will have a required pre-treatment biopsy (see Section 5.4) to assess the tumor micro-environment and level of T-cell infiltration. Therapy can be started before results of the biopsy are reported, as it will not be used to determine therapy.

On Day 1 of ***every other*** cycle (i.e., Cycle 1, Cycle 3, Cycle 5, etc.), patients will be given a dose of NT-I7 at the RP2D level by IM injection before receiving standard atezolizumab (1200 mg, intravenously). The NT-I7 dose will always be given at the RP2D level.

On Day 1 of ***every*** cycle (21-day cycle), patients will be given a dose of Atezolizumab (1200 mg, intravenously). Atezolizumab will be administered every 3 weeks (Q3W).

PK samples will be collected on all patients during the dose expansion phase of the study (refer to PK timepoints tables for specific draw times, Section 12.3.2).

CBC with differential including ALC will be checked at each cycle as standard of care. Research blood samples for T-cell phenotypes, CD4+ and CD8+ cell counts will be collected and tested at selected time points (see Sections 12.1.1 and 10).

Starting on Cycle 3, Day 1, NT-I7 administration will be skipped when the ALC is higher than 10,000 cells/μL. When automated and manual ALC counts are performed, manual ALC counts will be used if the results are conflicting. A blood draw will be performed prior to dosing on Day 1. The results will determine if NT-I7 should be interrupted for the current cycle. Treatment with atezolizumab will continue every 3 weeks.

Treatment with both study agents may continue for *up to 2 years* (see Section 6.4).

Patients will have a required *post-treatment biopsy* (see Section 5.4) to assess changes in the tumor micro-environment and the level of T-cell infiltration.

5.4 Other Procedures: Biopsy (core needle, punch or surgical excision)

A pre-treatment biopsy/tissue collection will be obtained within 14 days prior to the first dose of NT-I7 (Cycle 1, Day 1).

Note: If an archival tumor sample (at least 0.5 × 0.5 × 0.5 cm) is available for an enrolled patient, the pre-treatment biopsy is not required. The tumor sample must have been obtained ***after*** the last therapy.

A post-treatment tumor biopsy/tissue collection will be obtained between Cycle 2 Day 8 and Cycle 2 Day 21. The preference is for a biopsy/tissue collection as close to the equivalent of Cycle 2, Day 15.

The biopsies will be done according to clinical site Standard Operating Procedures (SOPs).

Tumor biopsies will be paramount. The biopsies will confirm T-cell infiltration and other potential actionable causes of failure.

5.5 Contraception

Administration of atezolizumab may have an adverse effect on pregnancy and poses a risk to the human fetus, including embryo-lethality. NT-I7 has not been tested for reproductive toxicity yet and may expose the same risk.

A woman is of childbearing potential if she is postmenarcheal, has not reached a postmenopausal state (≥ 12 continuous months of amenorrhea with no identified cause other than menopause), and has not undergone surgical sterilization (removal of ovaries and/or uterus).

Women of childbearing potential (including women who have had a tubal ligation) must have a negative serum pregnancy test within 72 hours prior to Cycle 1, Day 1. A follow up pregnancy test will be performed at the 90-day Safety Follow up visit.

Women of childbearing potential and men must agree to remain abstinent (refrain from heterosexual intercourse) or use contraceptive methods that result in a failure rate of < 1% per year during the study treatment period and for at least 5 months (150 days) after the last dose of study agent(s), per eligibility criteria 7 and 8. Men must also agree to refrain from donating sperm.

- Examples of contraceptive methods with a failure rate of < 1% per year include: bilateral tubal ligation, male sterilization, and established proper use of hormonal contraceptives that inhibit ovulation, hormone-releasing intrauterine devices, and copper intrauterine devices.
- Hormonal contraceptive methods must be supplemented by a barrier method plus spermicide.
- The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not acceptable methods of contraception.

Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

6. STUDY TREATMENT

Study treatment is defined as any investigational treatment(s), marketed product(s), placebo, or medical device(s) intended to be administered to a subject according to the study protocol.

6.1 Study Treatments Administration

Reported AEs and potential risks are described in Section 8. Appropriate dose modifications are described in Section 7. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Treatment will be administered on an outpatient basis.

Table 3 – Study Treatment Details

Regimen Description					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
NT-I7	None specific for NT-I7, but the below can be given prior to NT-I7 dosing[1]	Dose in µg/kg[2]	IM	Day 1 of every other cycle (Q6W)	21 days
Atezolizumab	antihistamines or antipyretics/ analgesics[1]	1200 mg	IV	Day 1 of each cycle (Q3W)	21 days

Abbreviations: IM=intramuscular; IV=intravenous.

1 Not permitted for the first dose. Optional for subsequent infusions.

2 Refer to Section 5 for assigned dose level.

6.1.1 *NT-I7*

Patients will receive NT-I7 according to the following dose escalation schedule. Dose escalation will proceed within each cohort as described in Section 5.2.

The NT-I7 dose must be administered 60 (±10) minutes before atezolizumab on Day 1 of every other cycle.

NT-I7 will be injected intramuscularly. Guidelines for IM injection by the research nurse or investigator are described in Appendix C.

The dose of NT-I7 administered will be determined using the patient's weight obtained at the baseline (screening) evaluation. Dosing is by actual body weight. Weight must be re-assessed prior to each NT-I7 dosing; if the patient's weight changes +/- 10% from baseline (screening visit), NT-I7 dose volume will be recalculated based on the new weight measurement.

For obese patients (BMI ≥ 30), dosing will be determined by adjusted body weight. Proceed with the following steps:

1. **Determine Ideal Weight (1 kg = 2.2 lbs):**
   Males: 50 kg + 2.3 kg x (inch over 5 feet)
   Females: 45.5 kg + 2.3 kg x (inch over 5 feet)
   (Patients less than 5 feet: subtract 2.3 kg/inch under 5 feet)

2. **Determine Adjusted Body Weight**:
   Ideal Weight + 0.4 x (actual weight – ideal weight) = adjusted body weight

NT-I7 will be skipped when the ALC is higher than 10,000 cells/µL on Day 1 of each cycle, starting on Cycle 3. (Section 5.1).

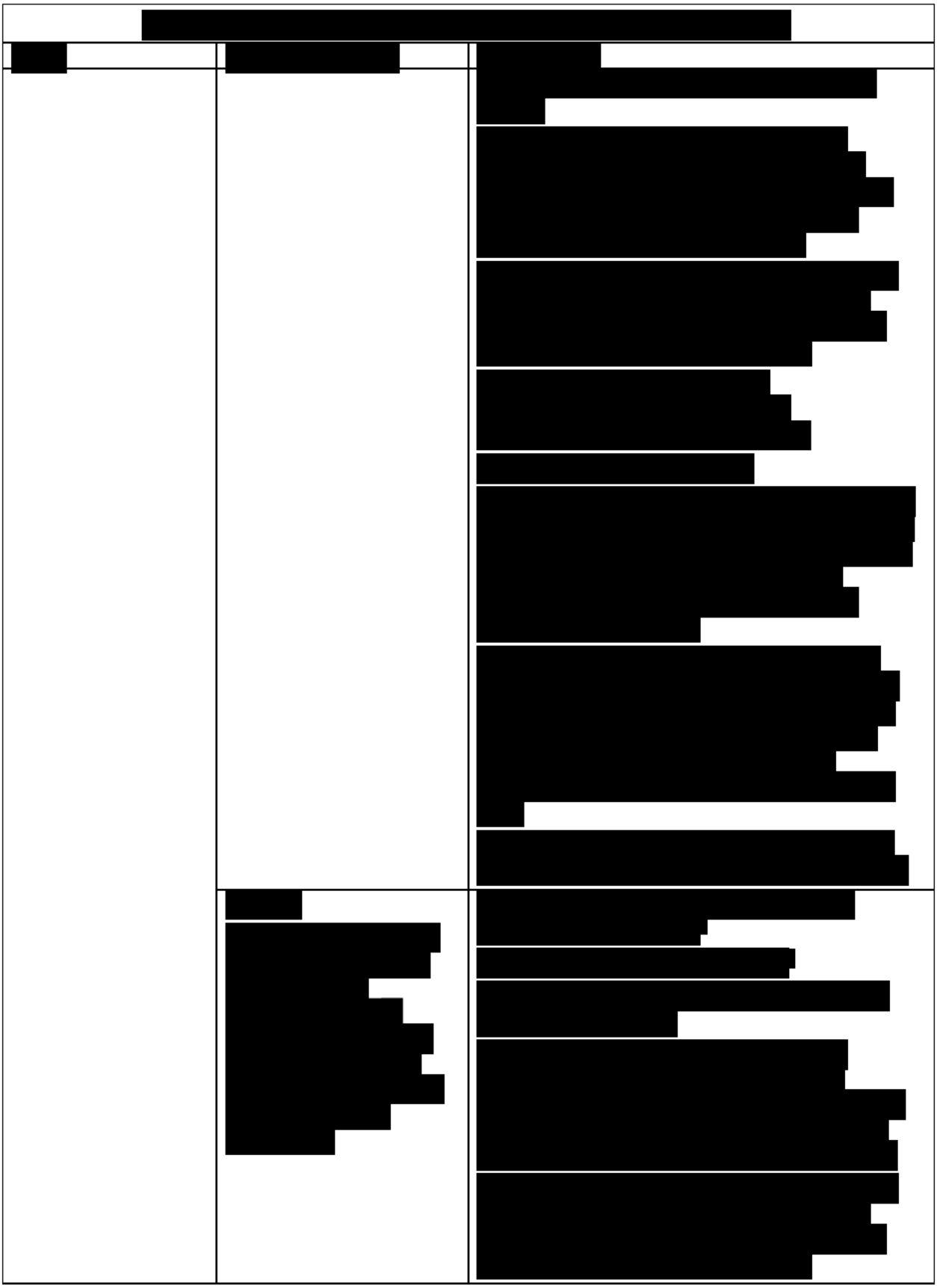
Syringes of NT-I7 will be prepared by the investigational pharmacy according to the dose assignment of the patient. Round doses to the nearest hundredth of an mL. Dose volumes greater than 1 mL may be divided into 2 or more injections (refer to Appendix C for dose preparation instructions).

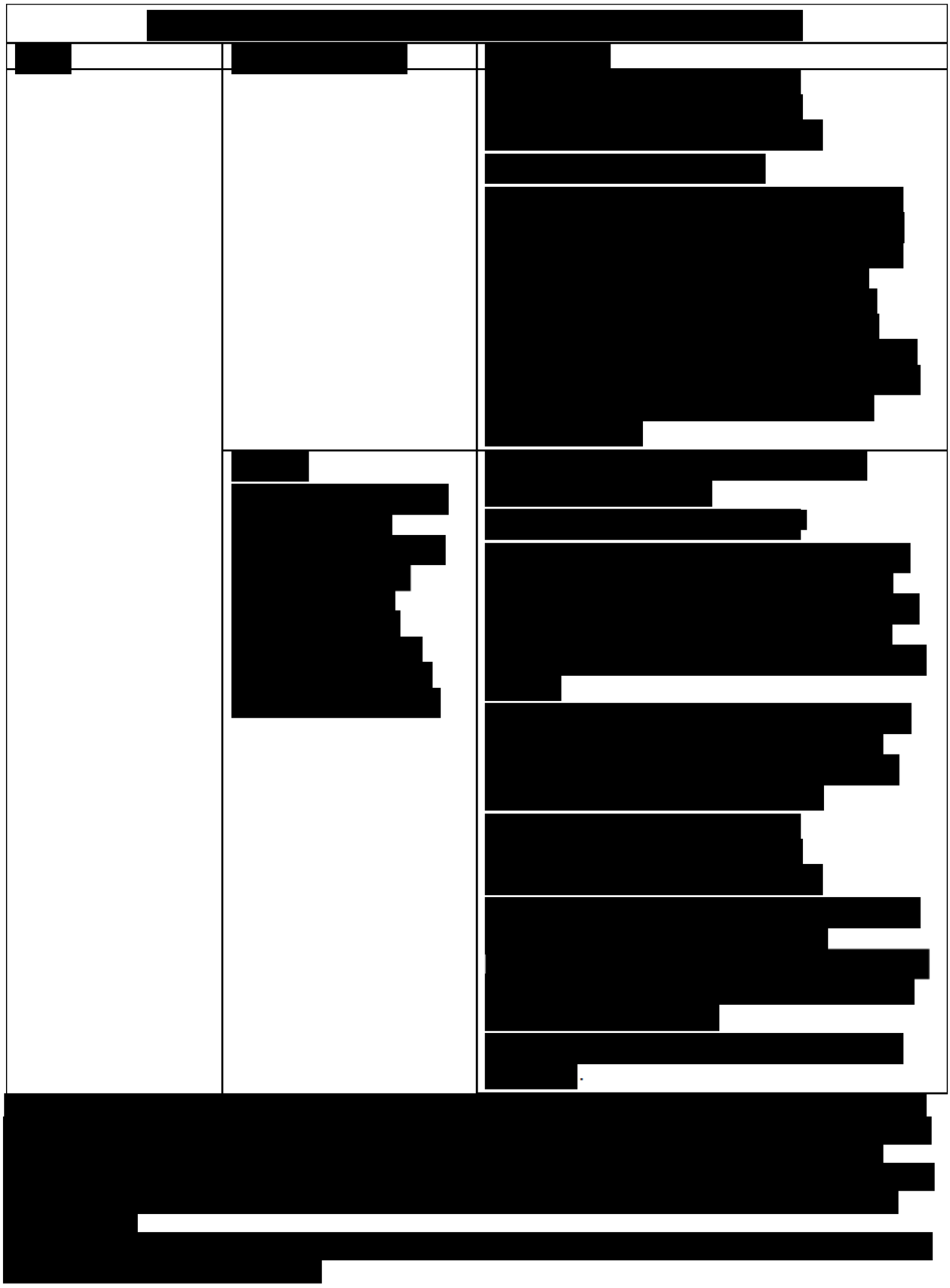
Patients will have their vital signs (heart rate, respiratory rate, blood pressure and temperature) determined within 60 minutes before the NT-I7 injection. Post-treatment vital signs will be monitored as described below (Section 6.1.2) following the guidelines for the atezolizumab infusion.

6.1.2 [redacted]

[redacted]

[redacted]





[redacted]

6.2 Dose-Limiting Toxicity

Dose-limiting toxicity is defined as any AE occurring within the first 21 days (i.e., Cycle 1, Day 1 through Day 21), that is considered to be at least possibly related to the combination study treatment (atezolizumab and NT-I7), and that meets at least one of the non-hematologic or hematologic criteria below.

Expected toxicities that are, in the opinion of the treating investigator, entirely attributable to atezolizumab will not be considered DLTs (following consultation with the protocol PI and/or ION Coordinating Center). The treating investigator should contact the ION Coordinating Center

for any questions concerning a DLT. The investigator, in consultation with the ION Coordinating Center and the protocol PI, will make the final decision.

6.2.1 *Hematologic toxicity*

- Any Grade 4 event, including laboratory electrolyte abnormalities, that is not easily reversed without sequelae
- Grade 4 neutropenia (Absolute Neutrophil Count [ANC] <500/mm^3) that does not improve to ≤Grade 3 within 48 hours
- Grade 3 febrile neutropenia
- Grade 4 thrombocytopenia (<25,000/mm^3) that does not improve to ≤Grade 3 within 48 hours
- Grade ≥3 thrombocytopenia (<50,000/mm^3) associated with Grade ≥3 bleeding or that requires transfusion therapy
- Grade ≥3 AST or ALT
- Grade ≥2 blood bilirubin or clinical jaundice
- Grade ≥2 AST or ALT and total bilirubin >2 x ULN with no initial findings of cholestasis (e.g., alkaline phosphatase ≤2 x ULN) and no other reason to explain the combination of elevated transaminases and bilirubin (e.g., viral hepatitis), i.e., Hy's law
- Any toxicity resulting in death (i.e., Grade 5)

Grade 3 lymphocyte count increased (>20,000 cells/µL) will not be reported as a DLT. An ALC of >20,000 cells/µL is a normal physiologic response to the study treatment.

Grade ≥3 lymphocytopenia will not be considered a DLT.

***Note**:* Peripheral lymphocytopenia after the first NT-I7 injection is not a sign of toxicity; it reflects the lymphocytes "homing effect" of NT-I7. Lymphocyte counts usually come back to baseline 5 to 7 days after the first injection.

6.2.2 *Non-hematologic toxicity*

Any Grade ≥3 non-hematologic toxicity (Common Terminology Criteria for Adverse Events [CTCAE] v5.0) will be considered a DLT, ***except*** for the following, which will not constitute DLTs:

- Grade ≥3 nausea, diarrhea or vomiting that resolves to Grade ≤2 in ≤72 hours with the use of adequate/maximal medical intervention and/or prophylaxis
- Grade 3 fatigue that resolves to Grade ≤2 in ≤7 days
- Grade 3 fever
- Grade 3 injection site reaction (unless operative intervention is required)
- Grade 3 arthralgia that can be adequately managed with supportive care or that resolves to Grade ≤2 within ≤72 hours
- Grade 3 hyperglycemia lasting ≤72 hours with standard antidiabetic therapy

- Grade 3 autoimmune thyroiditis or other disorder that can be managed by endocrine therapy that would not necessitate initiation of systemic corticosteroids (except for mineralocorticoids (e.g., fludrocortisone) for adrenocortical insufficiency)
- Grade ≥3 amylase or lipase that is not associated with symptoms or clinical manifestations of pancreatitis and resolves to ≤ Grade 1 within 7 days
- Grade ≥3 electrolyte abnormalities that last ≤72 hours, is not clinically complicated, and resolves spontaneously or responds to conventional medical interventions
- Clinical laboratory abnormalities that are reversible to ≤Grade 1 within 72 hours with outpatient care and/or monitoring, and are not considered clinically significant by the treating physician

Management and dose modifications associated with the above AEs are outlined in Section 7.

Once all patients in a cohort have completed the 3-week (21 days) DLT window, the AEs will be assessed by the Data and Safety Monitoring Committee, which includes the PI(s), study Sponsor and the ION Coordinating Center.

The patients must complete the full 3-week DLT window to be considered evaluable for DLTs. Patients who discontinue from the study before completion of the full 3-week DLT window for reasons other than the occurrence of a DLT (e.g., withdrawal of consent, rapid tumor progression, death due to rapid tumor progression, AE that does not meet DLT criteria) will not be considered evaluable for DLTs and will be replaced.

All patients will be monitored for occurrence of DLT. Monitoring of all safety and toxicity data is done by the protocol PI, the study Sponsor and the ION Coordinating Center on a real-time basis as data are entered into the electronic data capture (EDC) system. All participating sites must notify the protocol PI and the ION Coordinating Center when a DLT has occurred.

6.3 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of atezolizumab and NT-I7 with other concomitantly administered drugs, the case report form (CRF) must capture the concurrent use of all other drugs, over-the-counter medications or alternative therapies. The PI should be alerted if the patient is taking any agent known to affect or with the potential for drug interactions. The study team should check a frequently updated medical reference for a list of drugs to avoid or minimize usage.

[redacted]

6.4 Duration of Therapy

In the absence of treatment delays due to AE(s), treatment with atezolizumab and NT-I7 may continue for *up to 2 years* relative to the date of the 1st dosing or until one of the following criteria applies:

- Disease progression warranting alternative systemic therapy,

  ***Note**:* Patients with RECIST-defined progressive disease (PD) who are otherwise stable without symptomatic progression may continue study treatment until the next radiographic imaging time point (no less than 4 weeks after the prior assessment of PD) to assess for possible pseudo-progression.

- Intercurrent illness that prevents further administration of treatment,
- Unacceptable AE(s),
- Patient decides to withdraw from the study,
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator,
- Patient noncompliance,
- Dosing interruption lasting >12 weeks (see Section 7.1.1),
- Pregnancy
  - All women of child-bearing potential should be instructed to contact the investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation.

  - The investigator must immediately notify the ION Coordinating Center in the event of a confirmed pregnancy in a patient participating in the study.

- Termination of the study by sponsor(s), or
- The drug manufacturer can no longer provide the study agent

The reason(s) for protocol therapy discontinuation, the reason(s) for study removal and the corresponding dates must be documented in the CRF.

All patients who tolerate the protocol regimen and with either CR, partial response (PR) or SD will be permitted to continue study treatment until confirmed disease progression or for up to 2 years. Patients in response will have the option of continuing on atezolizumab using commercially purchased agent.

Discontinuation of treatment may be considered, at the discretion of the treating physician, for patients who have attained a confirmed CR that have been treated for at least 24 weeks with study treatment and had at least 2 treatments beyond the date when the initial CR was declared.

6.5 Study Treatment beyond Disease Progression

Immunotherapeutic agents such as atezolizumab may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents and can manifest as a clinical response after an initial increase in tumor burden or even the appearance of new lesions.

Patients who are clinically stable (without symptomatic progression) at an initial RECIST 1.1-defined PD may continue trial treatment as long as they meet all the following criteria (following consultation with the Protocol PI and/or ION Coordinating Center):

- Investigator-assessed clinical benefit
- Tolerance of study treatment
- Absence of signs and symptoms (including worsening of laboratory values) indicating disease progression
- No decline in ECOG performance status
- Absence of rapid progression of disease or threat to vital organs
- Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention

Patients should remain on the trial and continue to receive monitoring according to the Study Calendar.

After the initial RECIST 1.1-defined PD, a follow-up scan is to be collected at the next scheduled imaging visit (no less than 4 weeks after the prior assessment of PD). This scan is evaluated using the Confirmation of Radiological Progression criteria outlined in Section 11.1.4.

However, patients will not be permitted to continue study treatment if progression occurs after confirmed response (CR or PR as defined by RECIST 1.1) to study treatment in the target lesions

(regardless of the appearance of new lesions), i.e., the response and progression events both occurred in the target lesions while receiving study treatment during the same treatment period.

6.6 Duration of Follow-Up

The active study will end when the last patient completes the 90-day Safety Follow-up visit, approximately 27 months after enrollment.

End of treatment (EOT) visit should be performed 30 (± 5) days after the last administration of either agent or immediately before initiation of any other cancer therapy. At the EOT visit, tumor assessments are only required if not performed within the past 9 weeks (for patients who are on the study for ≤ 18 weeks) or 12 weeks (for patients who are on the study for > 18 weeks).

Safety Follow-up visit should be performed 60 (± 7) days and 90 (± 7) days after the last administration of either agent.

Patients who permanently discontinue study treatment for reasons other than objective RECIST disease progression should continue to have RECIST scans performed every 12 weeks (± 1 week), at the investigative site or locally, up to 12 months after discontinuation of study treatment. Standard of care disease assessments will be collected until the start of a new anticancer treatment, disease progression, pregnancy, death, withdrawal of consent or end of study, whichever occurs first.

After disease progression or start of new anticancer treatment, patient will be followed for survival every 12 weeks (±1 week) until death, loss to follow-up, withdrawal of consent or the end of the study, whichever occurs first. Survival follow-up can be done either by in-person visit or by telephone assessment.

Patients removed from study for unacceptable AE(s) will be followed until resolution or stabilization of the AE; in addition, the patients will be followed for disease status and overall survival, as described above.

6.7 Imaging Regimen

Imaging is based on standard of care practices, although the following is highly recommended.

All patients with known disease below the head and neck will have at least a diagnostic CT scan of the chest, abdomen and pelvis (CAP) performed within 28 days before the initiation of study treatment, 9 ± 1 weeks interval for the first two timepoints after initiation of treatment, and subsequent imaging performed at least every 12 ± 1 weeks as per standard of care for radiologic and tumor measurement.

If the patient has known disease in the head and neck, the same protocol will apply with the addition of a head and neck CT within 28 days before the initiation of study treatment, 9 ± 1 weeks interval for the first two timepoints after initiation of treatment, and subsequent imaging performed at least every 12 ± 1 weeks as per standard of care.

Finally, for melanoma patients, an MRI of the brain will be performed 9 ± 1 weeks interval for the first two timepoints after initiation of treatment, and subsequent imaging performed at least every 12 ± 1 weeks as per standard of care.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion pre-treatment and post-treatment.

6.8 Patient Replacement

All patients who receive at least 1 dose of the investigational treatment will be considered evaluable for safety and included in the overall safety analysis.

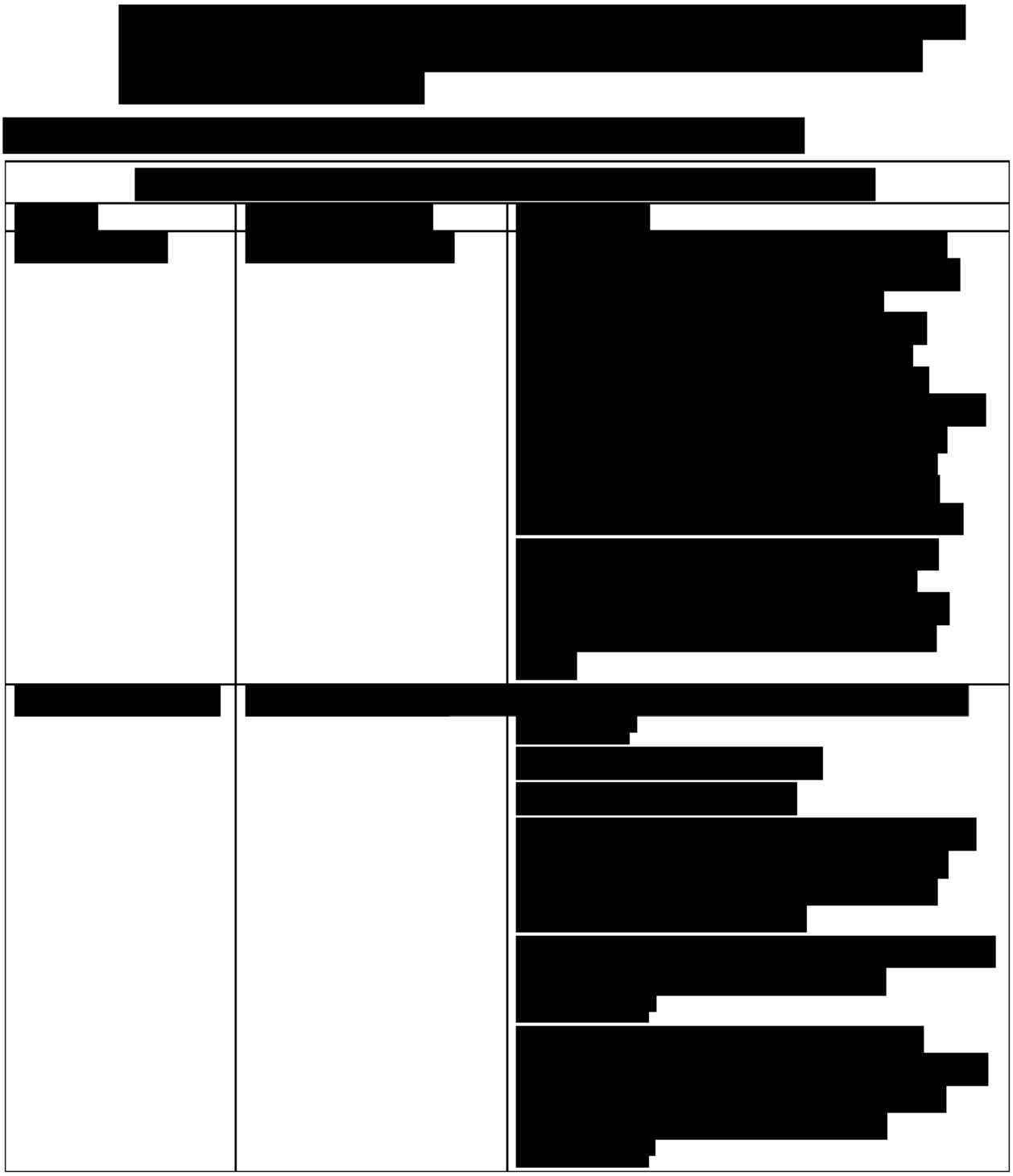
Patients who discontinue from the study before completion of the full 3-week DLT window for reasons other than the occurrence of a DLT (e.g., withdrawal of consent, rapid tumor progression, death due to rapid tumor progression, AE that does not meet DLT criteria) will not be considered evaluable for DLTs and will be replaced.

7. DOSING DELAYS/DOSE MODIFICATIONS

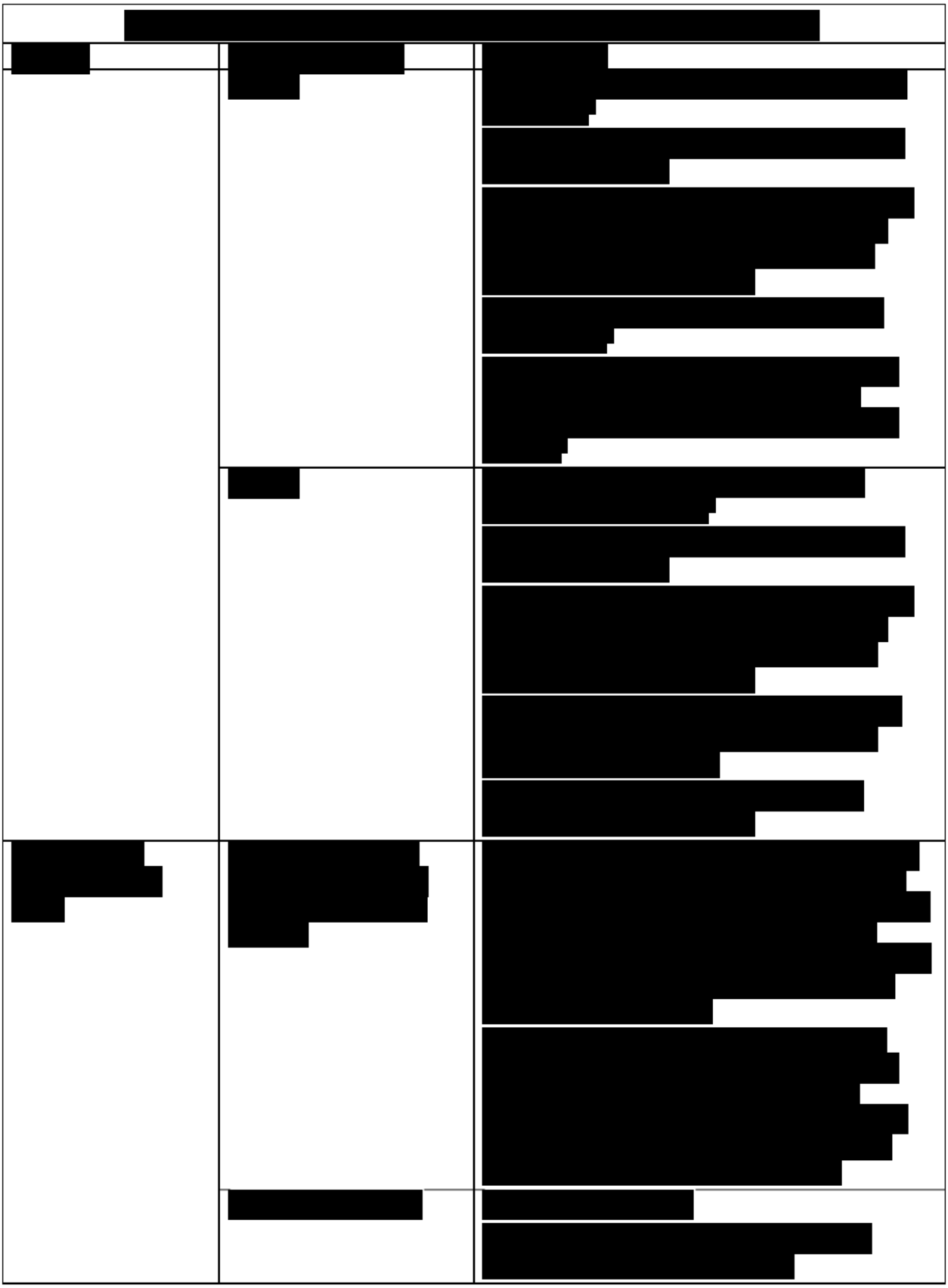
The National Cancer Institute (NCI) CTCAE Version 5.0 will be used to grade AEs. Patients enrolled in this study will be evaluated clinically and with standard laboratory tests before and at regular intervals during their participation in this study as specified in this section.

Patients will be evaluated for AEs (all grades), SAEs and AEs requiring study drug interruption or discontinuation at each study visit for the duration of their participation in the study.

potential risk when given in combination with other immunomodulating agents. Systemic




and contact Medical Monitor. c



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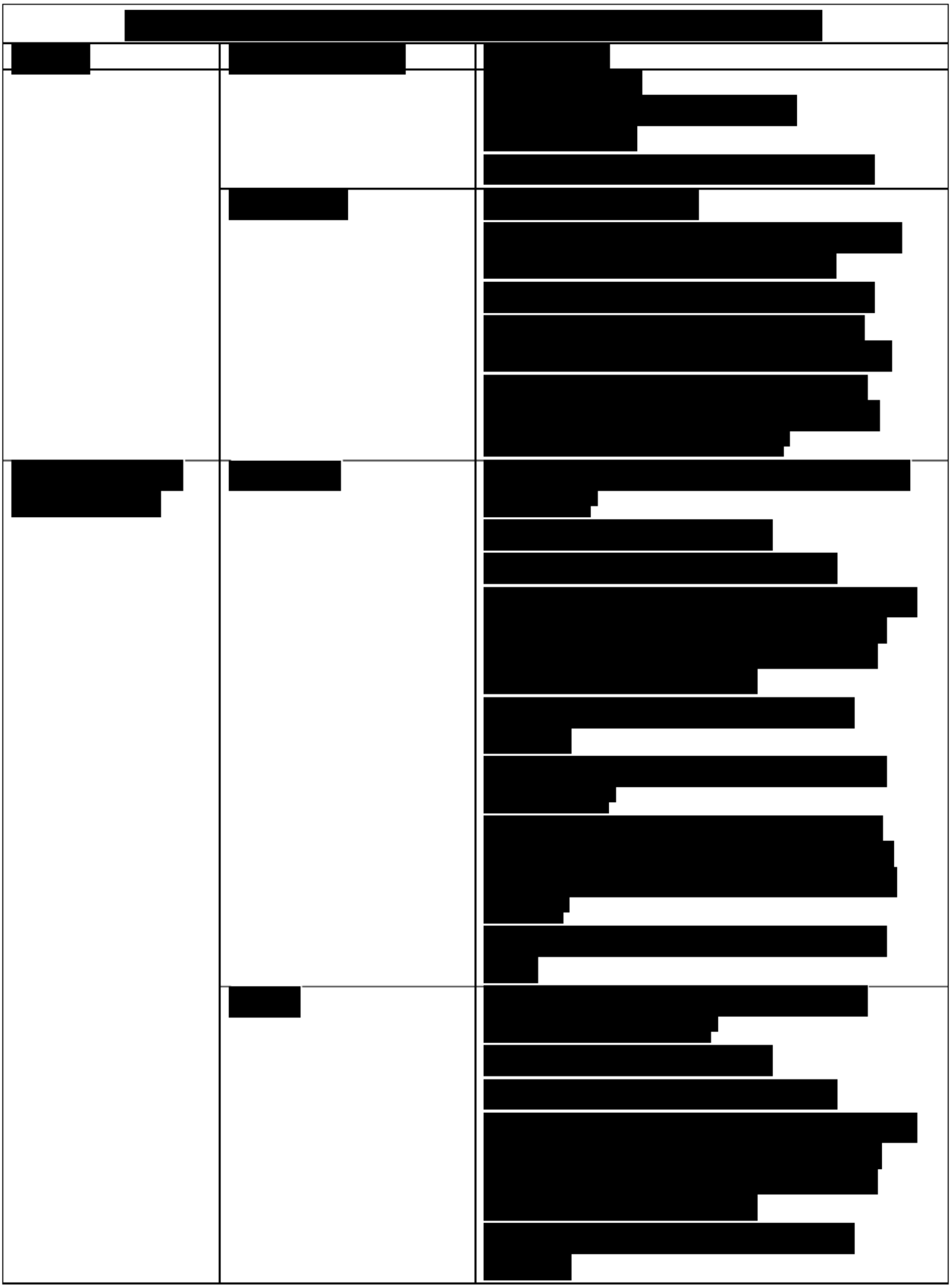
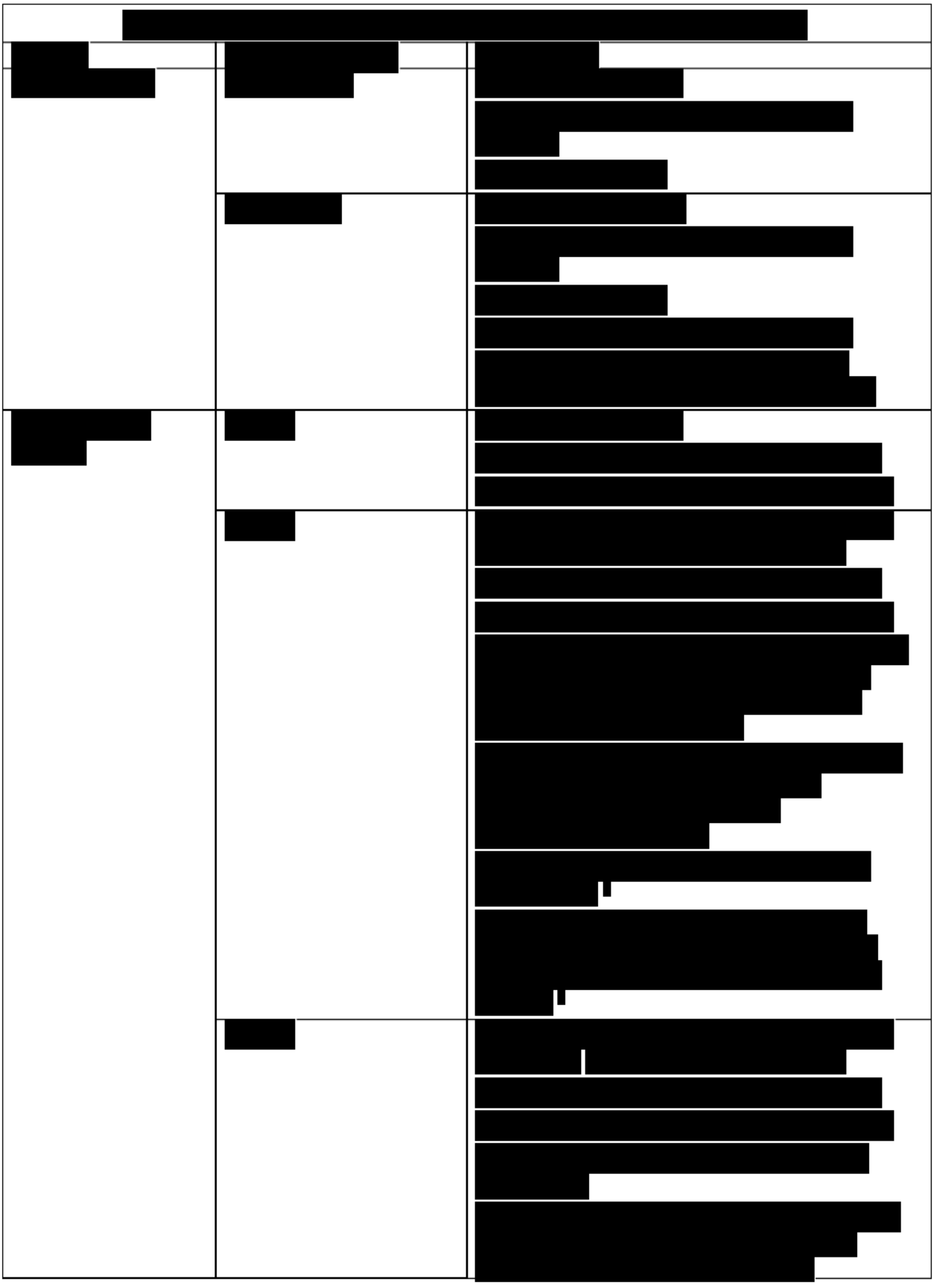
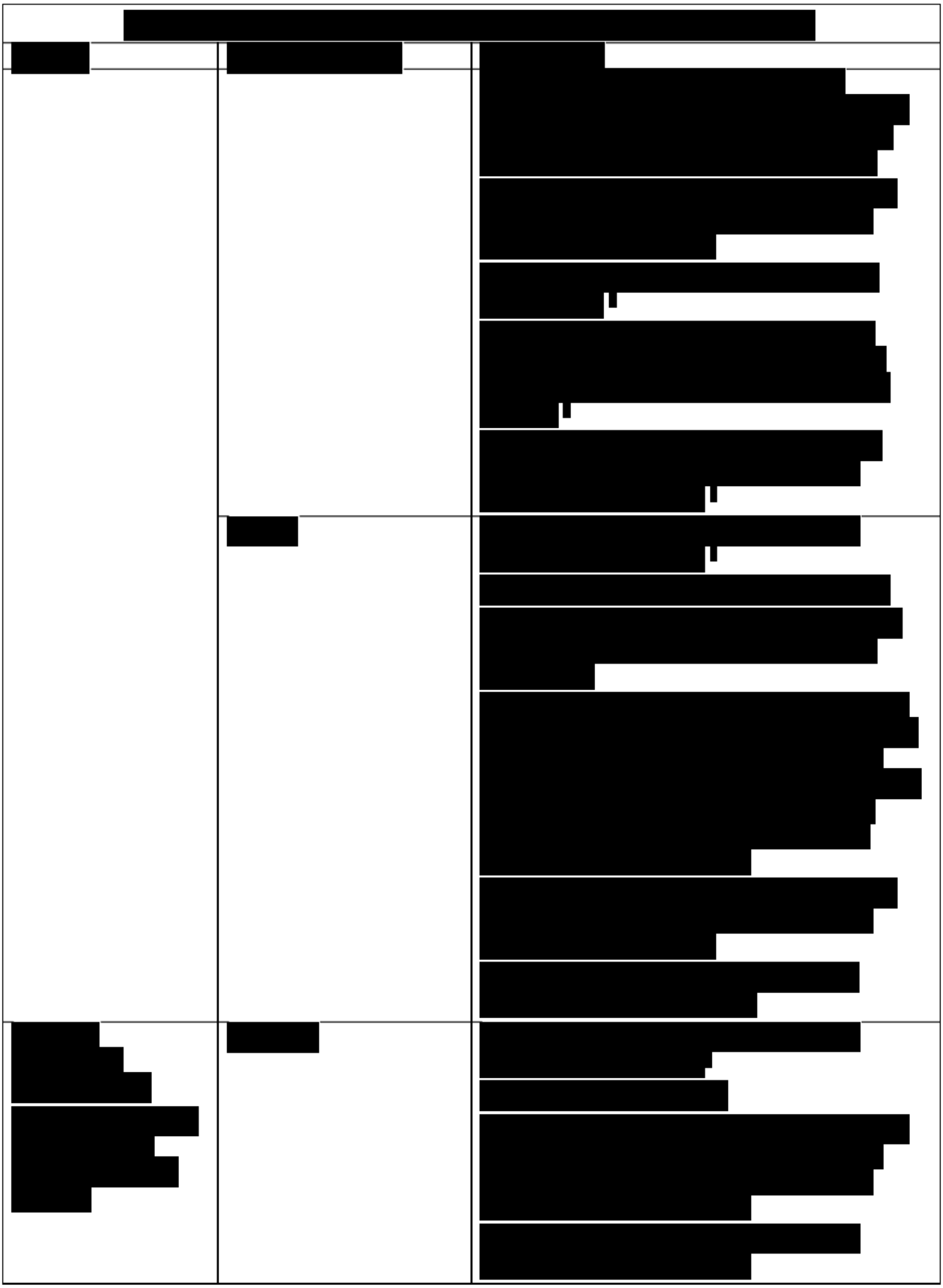
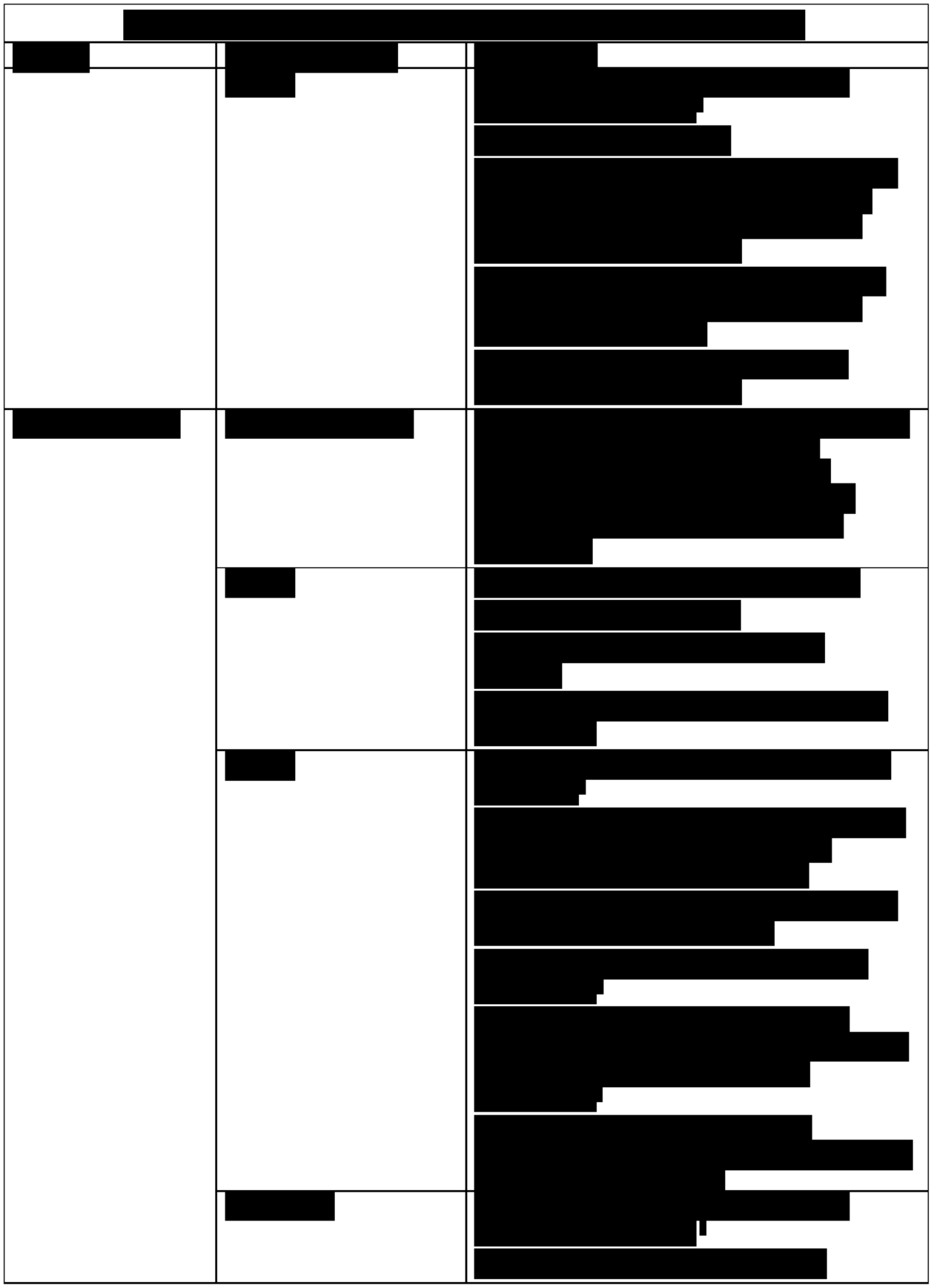
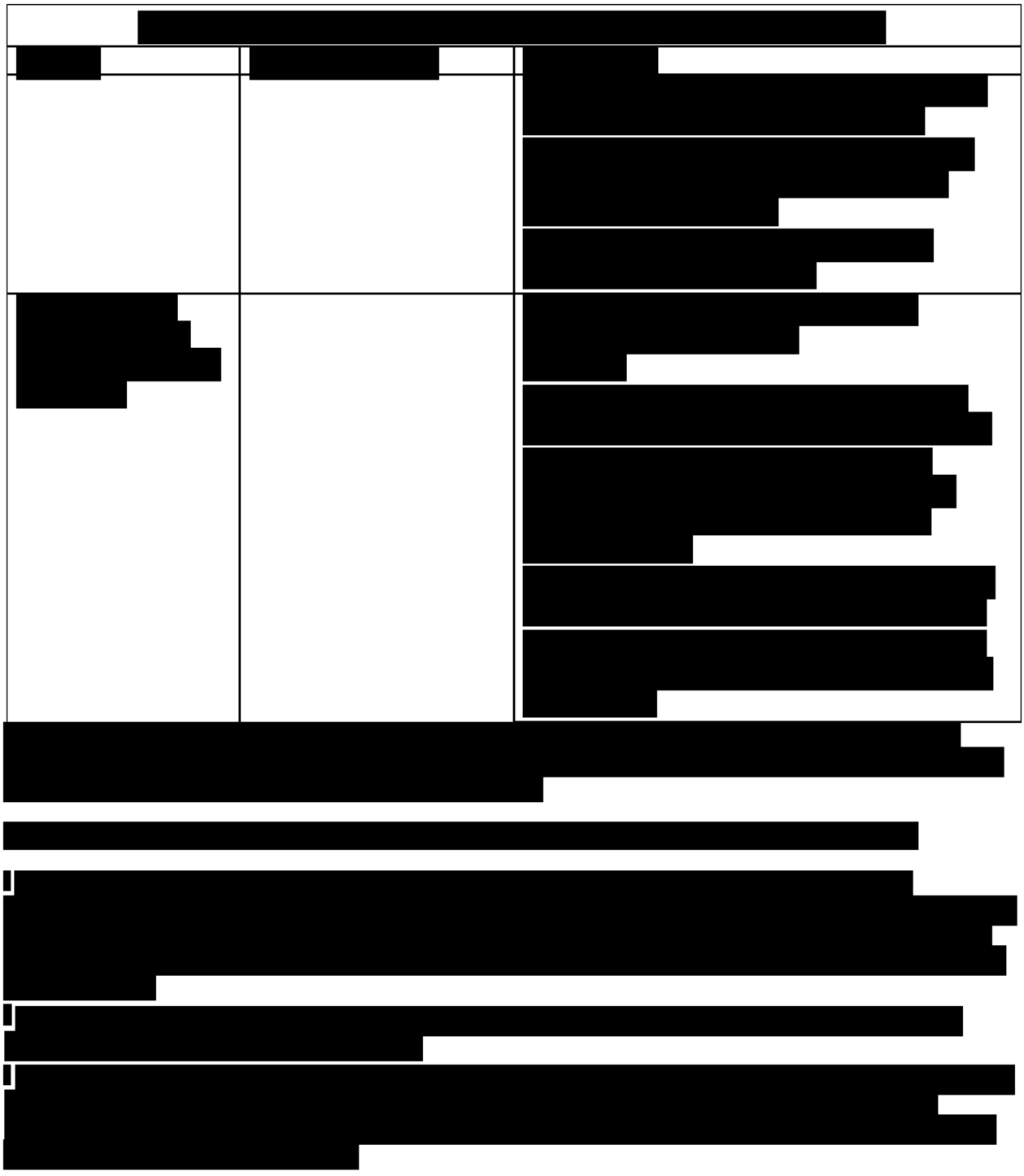










7.2 NT-I7 (rhIL-7-hyFc)

NT-I7 will be administered by IM injection Q6W in combination with atezolizumab. NT-I7 administration will be skipped when the ALC is higher than 10,000 cells/µL on Day 1 of each cycle, starting on Cycle 3. The acceptable length of interruption will be at the discretion of the protocol PI in consultation with the ION Coordinating Center and the NeoImmuneTech's Chief Medical Officer or designee.

Clinical experience has demonstrated that development of autoimmune inflammatory conditions is a general risk with therapeutics intended to enhance anti-tumor T-cell responses. Such irAEs have been described for virtually all organ systems and include, but are not limited to, colitis, hepatitis, pneumonitis, endocrinopathy, ocular toxicity, pancreatic toxicity and rash.

8. ADVERSE EVENTS: ASSESSING AND REPORTING REQUIREMENTS

An AE is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, and which does not necessarily have to have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product or protocol-specified procedure, whether or not considered related to the medicinal product or protocol-specified procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the study product is also an AE.

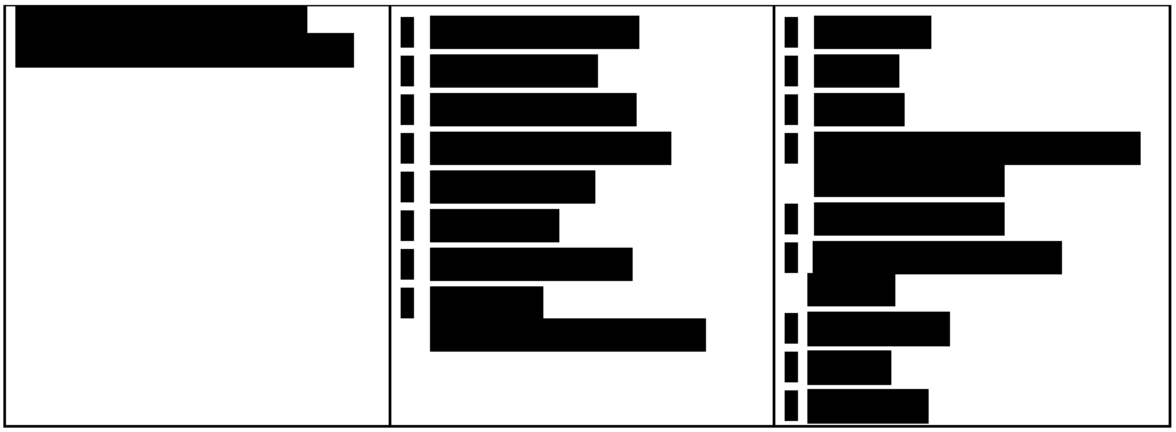
All AEs that occur on study, within 30 days of the last administration of the study treatment, must be reported. The reporting timeframe for AEs meeting the criteria of SAE is described in Section 8.3.1.

AEs are reported in a routine manner during the trial using the EDC system. Additionally, certain AEs must be reported in an expedited manner for timelier monitoring of patient safety and care. The following list of AEs (Section 8.1) and the characteristics of an observed AE (Section 8.2 and 8.3) will determine whether the event requires expedited reporting to the ION Coordinating Center.

Electronic monitoring of AEs will be done in the following ways: through the EDC system by the ION Coordinating Center, through email reporting by each clinical site to the ION Coordinating Center and by onsite monitoring of the clinical sites. Per GCP, all sites must enter data in a timely manner. AEs meeting the criteria of SAE will be triggered in the EDC system and email notification will be sent to the ION Coordinating Center for immediate review and distribution, as required by regulatory authorities.

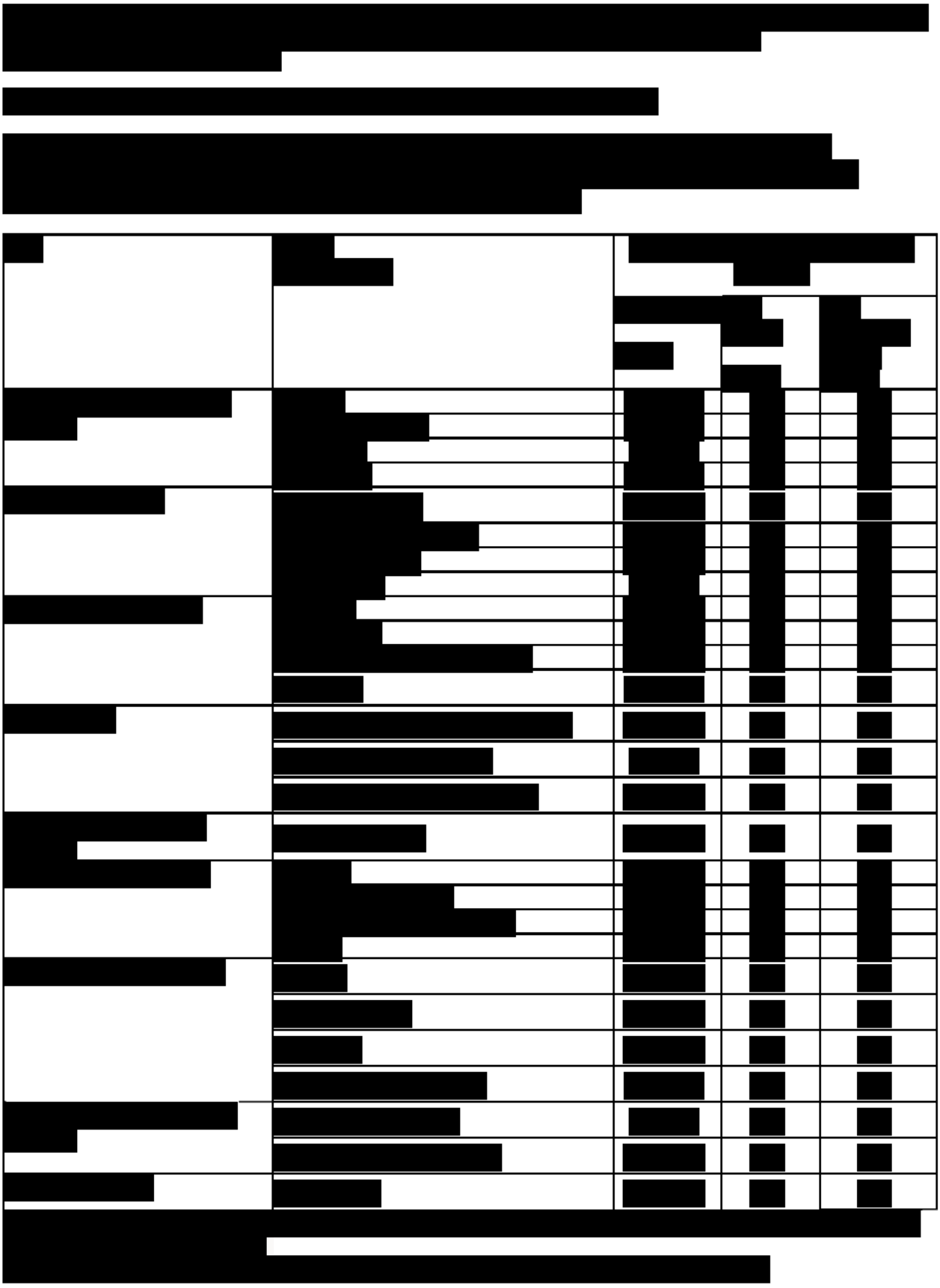
The investigator or qualified designee will assess each patient to evaluate for potential new or worsening AEs at the time points specified in the Study Calendar and more frequently if clinically indicated.

8.1 Adverse Events and Potential Risks List



8.1.2

[redacted]



Serious Adverse Drug Reactions for NT-I7 from Pooled Combination Therapy Clinical Trials

[redacted]

[redacted]

8.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade**: The descriptions and grading scales found in the CTCAE version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the Cancer Therapy Evaluation Program (CTEP) website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.
- **For expedited reporting purposes only**:
  - AEs of Special Interest for the protocol are outlined in Sections 8.3.2 and 8.3.3.
- **Attribution** of the AE:

- Definite – The AE is *clearly related* to the study treatment.
- Probable – The AE is *likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unlikely – The AE is *doubtfully related* to the study treatment.
- Unrelated – The AE is *clearly NOT related* to the study treatment.

8.3 Expedited Adverse Event Reporting

Expedited AE reporting for this study must be done by notifying the ION Coordinating Center, the study sponsor, NeoImmuneTech and IQVIA ***within 24 hours*** of the Investigator knowledge of the event via the study EDC system. The specific requirements are briefly outlined in the table below (Section 8.3.1).

In the event that access to the EDC system is disrupted (i.e., system is down/non-functional), the 24-hour notification is to be made to IQVIA Safety Operations via email at [redacted]). In the rare occurrence when Internet connectivity is lost, and the 24-hour notification cannot be made by email, the notification is to be made by fax at 1-[redacted] or by phone at [redacted]. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into the data capture system by the original submitter at the site.

8.3.1 *Expedited Reporting Guidelines*

Use the study protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

***Note*: A death occurring within 30 days of the last administration of study agent requires both routine and expedited reporting, regardless of causality. Attribution to treatment or other cause must be provided.**

Death due to PD should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention[1]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately (within 24 hours) report ANY Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 Code of Federal Regulations [CFR] 312.64)
An AE is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening AE
3) An AE that results in inpatient hospitalization or prolongation of existing hospitalization for ≥24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

ALL SERIOUS adverse events that meet the above criteria MUST be immediately reported to the ION Coordinating Center, NIT and IQVIA via electronic submission within the timeframes detailed in the table below.

Hospitalization	Grade 1 and Grade 2 Timeframes	Grade 3-5 Timeframes
Resulting in Hospitalization ≥24 hrs	24-Hour, 8 Calendar Days	24-Hour, 3 Calendar Days
Not resulting in Hospitalization ≥24 hrs	Not required	

Expedited AE reporting timelines are defined as:

- "24-Hour; 3 Calendar Days" - The AE must initially be submitted electronically within 24 hours of learning of the AE, and responses to related Safety Queries submitted no later than 3 calendar days after the initial 24-hour report.
- "24-Hour; 8 Calendar Days" – The AE must initially be submitted electronically within 24 hours of learning of the AE, and responses to related Safety Queries submitted no later than 8 calendar days after the initial 24-hour report.

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-hour notification and responses to related Safety Queries submitted within 3 calendar days for:

- All Grade 3, 4, and Grade 5 AEs

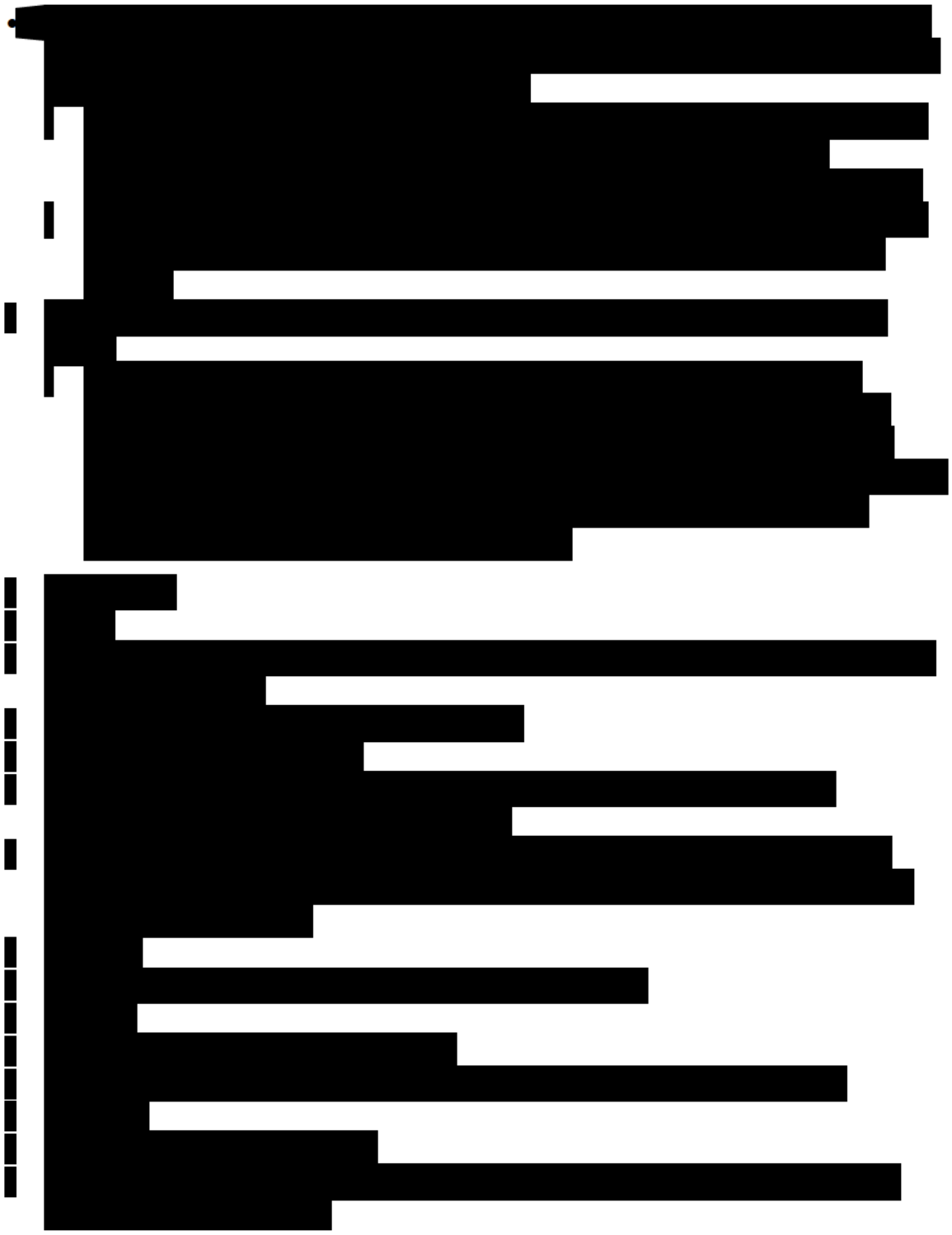
Expedited 24-hour notification and responses to related Safety Queries submitted within 8 calendar days for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

Abbreviations: AE=adverse event; CFR=Code of Federal Regulations; FDA=Food and Drug Administration; ICH=International Council for Harmonisation; IME=important medical events; NIT=NeoImmuneTech.

8.3.2 Adverse Events of Special Interest in Atezolizumab Studies

The following AEs are considered of special interest in patients receiving atezolizumab and must be reported expeditiously to the ION Coordinating Center, the study sponsor, NeoImmuneTech and IQVIA irrespective of regulatory seriousness criteria:



8.3.3 *Adverse Events of Special Interest in NT-I7 Studies*

The following AEs are of special interest in patients receiving NT-I7 and must be reported by the investigator expeditiously to the ION Coordinating Center, the study sponsor, NeoImmuneTech and IQVIA irrespective of regulatory seriousness criteria:

- [redacted]

8.4 Routine Adverse Event Reporting

All AEs **must** be reported in routine study data submissions. **AEs reported expeditiously to the sponsor and the ION Coordinating Center must <u>also</u> be reported in routine study data submissions.**

AE data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. For this trial the Adverse Event CRF is used for routine AE reporting.

8.5 Pregnancy

[redacted]

8.6 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

All secondary malignancies that occur after treatment with the study agents must be reported to the ION Coordinating Center, the study sponsor, NeoImmuneTech and IQVIA within ***24 hours*** of the investigator's knowledge of the event via the study EDC system. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (e.g., acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

8.7 Second Malignancy

A *second malignancy* is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine AE reporting unless otherwise specified.

9. PHARMACEUTICAL INFORMATION

A list of the AEs and potential risks associated with the investigational agents administered in this study can be found in Section 8.1.

9.1 [redacted]

[redacted]

conducted or are planned. There are no known interactions with other medicinal products or [redacted]

[redacted]

[redacted]

[redacted]

[redacted].

9.2 NT-I7

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

9.3 Agent Ordering and Agent Accountability

The investigator or those named as sub-investigators on the Statement of Investigator Form 1572 agree to supply study drugs only to those subjects enrolled in the study. The investigator or designee will keep a current and accurate inventory of all clinical drug supplies provided by the contract distributor designated by NeoImmuneTech.

Atezolizumab and NT-I7 are supplied by NeoImmuneTech. The study agents will be shipped from the designated contract distributor to each participating site (See Pharmacy Manual for instructions on how to order NT-I7). Please allow 5 days from the receipt of the drug order at NeoImmuneTech for drug shipment.

9.3.1 *Agent Inventory Records*

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing and final disposition of all agents received from NeoImmuneTech using the Investigational Agent (Drug) Accountability Record. Store and maintain separate Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol. Please refer to the Pharmacy Manual for the form to use and instructions on how to complete it.

9.3.2 *Investigator Brochure Availability*

The current versions of the IBs for the agents will be distributed to site investigators and research staff by the ION Coordinating Center.

9.3.3 *Useful Links and Contacts*

- ION Coordinating Center [redacted]

10. STUDY CALENDAR

Baseline evaluations (screening visit) are to be conducted within 2 weeks before the initiation of study treatment. Scans and x-rays must be done ≤4 weeks (28 days) before the initiation of study treatment. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours before the initiation of the next cycle of therapy. Patients will be assessed for pulmonary signs and symptoms throughout the study.

ARM I AND ARM II															
Treatment Cycle/Visit	**Screening**	**Cycle 1**			**Cycle 2**		**Cycle 3**		**Cycle 4**	**Cycle 5**	**Subsequent Cycle** (Repeat up to 2 year)	**End of Tx**	**Post-Treatment Follow-up**		
												30 days ± 5 days post Tx	Safety FU	Disease Assessment[g]	Survival FU
Scheduling Window	Within 14 days	Day 1	Day 2	Day 8± 2 days	Day 1± 2 days	Day 8± 2 days	Day 1± 2 days	Day 8± 2 days	Day 1± 2 days	Day 1± 2 days	Day 1± 2 days		60 and 90 days ± 7 days post Tx	Every 12 wks ± 1 wk	Every 12 wks ± 1 wk
NT-I7*		A					A			A	A				
Atezolizumab		B			B		B		B	B	B				
Administrative procedures															
Informed consent	X														
Demographics	X														
Medical history	X														
Concurrent meds	X	X----------									----------X	X	X	X	X
Clinical Procedures/Assessments															
Physical exam	X				X		X		X	X	X	X	X		
Vital signs[a]	X	X		X	X		X	X	X	X	X	X	X		
Height	X														
Weight	X	X			X		X		X	X	X	X	X		
AEs evaluation		X----------									----------X	X	X	X	
Immune-related AEs evaluation		X----------									----------X	X	X	X	
Performance status	X	X		X	X		X	X	X	X	X	X	X		
EKG	X	X[1] (as indicated) ----------									----------X[1]				
Laboratory Assessments (Safety Labs)															
CBC w/diff, plts	X	X		X	X		X	X	X	X	X	X	X		
Serum chemistry[b]	X	X		X	X		X	X	X	X	X	X	X		
Pregnancy Test (serum)	X[c]												X[c]		
Hepatitis B testing	X														
INR and aPTT	X														

ARM I AND ARM II															
Treatment Cycle/Visit	**Screening**	**Cycle 1**			**Cycle 2**		**Cycle 3**		**Cycle 4**	**Cycle 5**	**Subsequent Cycle** (Repeat up to 2 year)	**End of Tx**	**Post-Treatment Follow-up**		
												30 days ± 5 days post Tx	Safety FU	Disease Assessment[g]	Survival FU
Scheduling Window	Within 14 days	Day 1	Day 2	Day 8± 2 days	Day 1± 2 days	Day 8± 2 days	Day 1± 2 days	Day 8± 2 days	Day 1± 2 days	Day 1± 2 days	Day 1± 2 days		60 and 90 days ± 7 days post Tx	Every 12 wks ± 1 wk	Every 12 wks ± 1 wk
Efficacy Measurements															
Tumor measurements	Within 28 days	Tumor measurements are performed at *9 weeks ± 1 week for the first two timepoints, then every 12 weeks ± 1 week*. Documentation (radiologic) must be provided for patients removed from study for progressive disease.										X[i]		X	
Radiologic evaluation	Within 28 days	Radiologic measurements should be performed at *9 weeks ± 1 week for the first two timepoints, then every 12 weeks ± 1 week*										X[i]		X	
Tumor Biopsies/Archival Tissue Collection															
Tumor Biopsy	X[d]					X[d]									
Correlative Studies Blood Draws[e]															
T-cell count (CD4/CD8)		X[e]			X[e]		X[e]		X[e]	X[e]	X[e]				
Pharmacokinetics[h]		X	X**	X			X			X		X			
Immunophenotyping		X		X[k]	X		X	X	X	X		X			
Multiplex Cytokines		X			X		X		X	X		X			
TCR sequencing		X			X		X		X	X					
ELISPOT		X			X		X		X	X					
Kyn/Trp and Arginine		X			X		X		X	X		X			
Immunogenicity Testing[j] (NT-I7)		X			X		X		X	X	X	X	X[j]		

A NT-I7: Dose as assigned; starting with DL4 (1200 µg/kg), NT-I7 dosing is changed from Q3W to Q6W (i.e., Cycle 1, Day 1, Cycle 3, Day 1, Cycle 5, Day 1, etc.). NT-I7 will be skipped when the ALC is higher than 10,000 cells/µL on Day 1 of each cycle, starting on Cycle 3 (protocol Section 5.2).

* NT-I7 must be administered 60 (± 10) minutes before atezolizumab.

B Atezolizumab: Dose as assigned; once every 3 weeks, starting Cycle 1, Day 1.

a Vital signs assessment before and after study treatment is required (Section 6.1.1 and 6.1.2).

b Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], lipase, amylase, TSH and sodium.

c Pregnancy test (women of childbearing potential) must be performed within 72 hours prior to initiation of study treatment per eligibility criteria and at the 90-day Safety Follow up visit.

d Tumor biopsy/tissue collection (fresh) must be obtained within 14 days prior to Cycle 1, Day 1. If an archival tumor sample is available, the pre-treatment biopsy is not required. Post-treatment tumor biopsy must be obtained between Cycle 2, Day 8 and Cycle 2, Day 21 (Section 5.4)

e To be performed **prior** dosing (NT-I7 and/or atezolizumab)

g Disease assessment per standard of care

h To be collected on all patients (dose escalation and dose expansion); refer to the Pharmacokinetic timepoints tables for details (12.3.2); **Day 2 on Dose Escalation (Phase 1b) patients only, and is optional.

i Only required if not performed within the past 9 or 12 ± 1 weeks – Section 6.6

j Refer to Sections 11.2.3 and 12.3.1 for all details. If End of Treatment visit is ADA-positive, repeat testing at 90-day Safety Follow-up and again every 90 days, until no longer ADA-positive.

k To be collected on all patients (dose escalation and dose expansion).

l EKG must be obtained at Screening. As clinically indicated, EKGs are to be obtained to evaluate for cardiac toxicity during subsequent cycles of therapy and follow-up visits.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 9 weeks for the first two timepoints, then at least every 12 weeks as per standard of care.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised RECIST guideline (version 1.1) 48. RECIST 1.1 will be used as the primary measure for assessment of tumor response, date of disease progression, and as the basis for all protocol guidelines related to disease status (e.g., discontinuation of study treatment). Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

This study will also evaluate response and progression using immune-related RECIST (iRECIST) criteria. Investigators should note the different requirements for confirmatory scans as well as follow up of the two criteria.

11.1.1 *Definitions*

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with atezolizumab and/or NT-I7 until 30 days after the last dose of study treatment.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least 1 cycle of therapy and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression before the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least 1 cycle of therapy and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence or unequivocal progression of the lesions.

11.1.2 *Disease Parameters*

Measurable disease. Measurable lesions are defined as those that can be accurately measured in at least 1 dimension (longest diameter to be recorded) as ≥20 mm (≥2 cm) by chest x-ray or as ≥10 mm (≥1 cm) with CT scan, MRI or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

***Note**:* Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm (≥1.5 cm) in short axis when assessed by CT scan (CT scan slice thickness

recommended to be no greater than 5 mm [0.5 cm]). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm [<1 cm] or pathological lymph nodes with ≥10 to <15 mm [≥1 to <1.5 cm] short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease and abdominal masses (not followed by CT or MRI) are considered as non-measurable.

***Note**:* Cystic 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 noncystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. 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. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. 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 are to be included in the sum, then 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.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

11.1.3 *Methods for Evaluation of Measurable Disease*

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

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. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm (≥1 cm) diameter as assessed using

calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray. Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm (0.5 cm) or less. If CT scans have slice thickness greater than 5 mm (0.5 cm), the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g., for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts and diseases. Ideally, the same type of scanner should be used, and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT. At present, the low dose or attenuation correction CT portion of a combined positron emission tomography (PET)-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound. is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence after CR or surgical resection is an endpoint.

Tumor markers. Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete

clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) [49] and prostate-specific antigen (PSA) response (in recurrent prostate cancer) [50,51] have been published. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [52].

Cytology, Histology. These techniques can be used to differentiate between PR and CR in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or SD is mandatory to differentiate between response or SD (an effusion may be a side effect of the treatment) and PD.

FDG-PET. While fluorodeoxyglucose (FDG)-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

***Note**:* A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

11.1.4 *RECIST 1.1 Response Assessment*

11.1.4.1 *Evaluation of Target Lesions*

Complete Response (CR). Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm (<1 cm).

Partial Response (PR). At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD). At least a 20% increase in the sum of the 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 (0.5 cm). (Note: the appearance of 1 or more new lesions is also considered progressions).

Stable Disease (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.

11.1.4.2 *Evaluation of Non-Target Lesions*

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm [<1 cm] short axis).

***Note**:* If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of 1 or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of 1 or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or PI).

11.1.4.3 *Evaluation of Best Overall Response*

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	>4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	>4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once >4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR, or CR

Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	
* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion. ** Only for non-randomized trials with response as primary endpoint. *** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression. Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment.				

Abbreviations: CR=complete response; PD=progressive disease; PR=partial response; RECIST=Response Evaluation Criteria in Solid Tumors; SD=stable disease.

For Patients with Non-Measurable Disease (i.e., **Non-Target Disease)**

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
*'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

Abbreviations: CR=complete response; PD=progressive disease; SD=stable disease.

11.1.5 *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 best overall responses will be recorded separately.

Confirming progression: Unlike RECIST 1.1, iRECIST requires the confirmation of progression and uses the terms iUPD (unconfirmed progression) and 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 one 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 iSD, iPR, or iCR if those criteria are met compared to baseline). 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 (*Lancet Oncol* 18:e143-e152, 2017 - Table 2).

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.

Time-point (TP) iResponse

Target Lesions*	**Non-Target Lesions***	**New Lesions***	**Time Point Response**	
			No prior iUPD**	**Prior iUPD**, *****
iCR	iCR	No	iCR	iCR
iCR	Non-iCR/Non-iUPD	No	iPR	iPR

Time-point (TP) iResponse

Target Lesions*	**Non-Target Lesions***	**New Lesions***	**Time Point Response**	
			No prior iUPD**	**Prior iUPD**, *****
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	Yes	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.

Time-point (TP) iResponse

Target Lesions*	**Non-Target Lesions***	**New Lesions***	**Time Point Response**	
			No prior iUPD**	**Prior iUPD**, *****
* Using RECIST 1.1 principles. If no PSPD occurs, RECIST 1.1 and iRECIST categories for CR, PR, and SD would be the same. ** in any lesion category. *** previously identified in assessment immediately prior to this TP.				

All patients will have their iBOR from the start of study treatment until the end of treatment classified as outlined below.

iRECIST best overall response (iBOR)

TPR 1	**TPR 2**	**TPR 3**	**TPR 4**	**TPR 5**	**iBOR**
iCR	iCR, iPR, iUPD, NE	iCR, iPR, iUPD, NE	iUPD	iCPD	iCR
iUPD	iPR, iSD, NE	iCR	iCR, iPR, iSD, iUPD, NE	iCR, iPR, iSD, iUPD, iCPD, NE	iCR
iUPD	iPR	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
Table assumes a randomized study where confirmation of CR or PR is not required. • NE = not evaluable that cycle. • 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.					

11.1.6 *Duration of Response*

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR/PR or iCR/iPR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: SD is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.1.7 *Progression-Free Survival*

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

11.1.8 *Response Review*

Radiological images will be collected for a possible review of response by an expert(s) independent of the study. Measurement of tumor response by a trained radiologist/nuclear medicine physician at each clinical site will be chronicled and reported unless or until a central expert review takes place.

11.2 Correlative Studies

11.2.1 *Integrated Correlative Studies Background*

11.2.1.1 *Evaluation of Peripheral CD4+ and CD8+ T-cell Counts – Integrated Laboratory Correlative Study #1*

As described above, NT-I7 has been shown to double the number of peripheral blood lymphocytes including T-cells. Thus, CD4+ and CD8+ T-cell numbers are important biomarkers for the effect of NT-I7 on the peripheral T-cell populations. To assess the overall pharmacodynamic effect of atezolizumab plus NT-I7 and to determine whether NT-I7 in combination with atezolizumab results in the same, or different, level of expansion of peripheral lymphocytes, T-cell counts (both CD4+ and CD8+) will be evaluated in Clinical Laboratory Improvement Amendments (CLIA)-certified laboratories locally at each site. CD4+ and CD8+ T-cell counts will be evaluated at baseline, during treatment, and at end of therapy.

Hypothesis: The combination of atezolizumab with NT-I7 will result in similar levels of peripheral blood T-cell expansion as with NT-I7 alone.

11.2.1.2 *Slide-Based Immune Phenotype Panel -- Multispectral IHC – Integrated Laboratory Correlative Study #2*

Because tumor-infiltrating immune cells are associated with clinical outcomes, infiltrates from pre-treatment tumor tissue and from biopsies taken during treatment will be assessed by immunohistochemistry (IHC) to determine extent and the nature of T-cell infiltration and of the overall immune milieu of the TME.

Paired baseline and post-baseline tumor biopsies will be evaluated using quantitative multicolor IHC with Perkin-Elmer's Vectra IHC platform. This platform employs spectral deconvolution imaging to separate optical signals from each antibody together with state-of–the-art image analysis capabilities to facilitate robust quantitative slide-based immunophenotyping. Using the

Vectra multispectral imaging platform (PerkinElmer), adjacent tumor sections will be stained and imaged with a series of 5-6 antibodies per section simultaneously to investigate the spatial relationship between key immunomodulatory cell types and molecules. The antibodies will include but are not limited to the following: pancytokeratin (AE1/AE3), CD8+, CD4+, PD-1, PD-L1, CD68, CD163, FOXP3, arginase, and pan- major histocompatibility complex (MHC) class I and class II. Data will be generated in the form of total positive cells, positive cells/mm^2, percentage positive cells in a population, ratios of cells and molecules of interest (e.g., PD-L1:CD8+ or FOXP3+CD4+:CD8+). Additionally, tumors may also be assayed by IHC for a variety of other markers including CD80, IFN-γ CD3, CD5, TIA-1, CD20, CD11b, CD137, CD45RO, CD301CD56 and CD16 (NK and NK T-cells), CD79a (B cells), GITR, TIM-3, FOXP3, LAG3. Furthermore, quantitative analysis of tumor-infiltrating lymphocyte (TIL) distribution (i.e., stromal and interface/invasive margin) will be performed. Anti-PD-L1 immunohistochemical staining with the SP142 PD-L1 clone (IHC assay, HistoGeneX/CellCarta) kit will be performed in addition to the multispectral IHC (msIHC) to serve as both an independent test and control for the reproducibility of the msIHC assay. The study is intended to evaluate for changes in the immune profile of the TME. If sufficient archival tumor tissue collected before therapy is not available, a baseline biopsy will be obtained before enrolling in the study as part of this protocol.

IHC studies will be performed at the Fred Hutchinson Cancer Center (Fred Hutch) Immunopathology Laboratory, Seattle, WA, or other agreed upon investigator.

***Hypothesis**:* The combination of atezolizumab with NT-I7 will alter the TME toward a pro-inflammatory phenotype.

11.2.1.3 *Assessment of Tumor Biopsy by Gene Expression Analysis -- Interferon γ (IFNγ) Gene Expression Signature - NanoString® PanCancer IO 360TM Gene Expression Panel and the nCounter® PanCancer Immune Profiling Panel – Integrated Laboratory Correlative Study #3*

To identify potential signatures correlating with tumor T-cell infiltration patterns and clinical response, tumor tissue will be tested for gene expression using NanoString® technology on unstained tissue formalin-fixed paraffin-embedded (FFPE) slide material. Specifically, slides will be analyzed using a 770-gene panel (nCounter® PanCancer Immune Profiling Panel), which contains markers for 24 different immune cell types and populations, 30 common cancer antigens and genes that represent immune responses including checkpoint blockade genes as well as the NanoString® nCounter® PanCancer IO 360™ Gene Expression Panel, which contain genes associated with the IFNγ Gene Expression Signature. These gene expression profiles may provide an additional detailed phenotype to guide correlative investigation.

NanoString®-based gene expression analysis, specifically for the IFN γ Signature, will be used to test the hypothesis that the investigational study treatment regimen, NT-I7 plus atezolizumab, will promote a proinflammatory antitumor immune response. The IFNγ Signature, which is comprised of IDO1, CXCL10, CXCL9, HLA-DRA, STAT1 and IFNγ, has previously been shown to correlate with anti-PD-1 response and nonresponse. RNA will be extracted from all paraffin-embedded tumor samples and analyzed using the aforementioned NanoString® nCounter technology or similar panels. The samples used will be taken from tissue sections adjacent to

those stained for mIHC to facilitate comparison between these analysis sets. Samples will consist of matched pre- and post-treatment FFPE biopsies.

These analyses will be performed in coordination with NanoString® Technologies in Seattle, WA, and analyzed using qualified analytic tools at the Immunopathology Lab at Fred Hutch.

***Hypothesis**:* The combination of atezolizumab with NT-I7 will alter the TME toward a pro-inflammatory gene expression programming (GEP) phenotype, and distinct gene expression patterns will be identified that may correlate with clinical outcomes.

11.2.1.4 *T-Cell Receptor Repertoire Analysis -- TCR sequencing – Integrated Laboratory Correlative Study #4*

We will perform TCR sequencing where pre-treatment and post-treatment biopsies are available. TCR repertoire analysis will be performed on RNA extracted from FFPE biopsies and T-cells isolated from peripheral blood mononuclear cells (PBMCs) collected throughout study participation. TCR repertoire analysis has been standardized and commercialized by Adaptive Biosciences. The TCR sequences will also specifically be queried for sequences already known to encode for TCRs reactive to shared antigens in the particular tumors, such as MCC virus in virus positive MCC.

Samples will be analyzed at Adaptive Bioscience in Seattle, WA, or other agreed upon vendor or collaborator.

***Hypothesis**:* The combination of atezolizumab with NT-I7 will: i) result in emergence and/or increased prevalence of specific TCR sequences reflecting the clonal expansion of antitumor T-cells; and ii) expansion of those specific T-cell clones in TILs (exhibited by higher TCR clonality) will be associated with improved response to therapy.

11.2.1.5 *Tumor-Associated Neo-antigen Discovery -- Whole Exome Sequencing (WES) and RNAseq and Antigen Prediction – Integrated Laboratory Correlative Study #5*

In order to investigate the role of T-cell–mediated immunity directed toward tumor-specific mutant antigens, whole exome sequencing (WES) and RNAseq of tumor cells from baseline tumor biopsy relative to non-tumor cells from whole blood (PBMC) will be performed to: i) identify tumor-associated neoantigens; and ii) identify the genomic variances that may contribute to response or disease progression and provide an understanding of molecular abnormalities.

DNA and RNA will be extracted from baseline or archival FFPE tumor biopsies and then sequenced to identify overexpressed, known “shared” tumor-associated antigens (TAAs) and nonsynonymous expressed mutations using available and standard-of-practice technologies. For example, a computational approach (BIMAS and SYFPEITHI algorithms) can be utilized to analyze and prioritize these mutation-derived “virtual” antigens in terms of their likelihood to bind to/be presented by relevant MHC I alleles. Potential antigens can be encoded in tandem minigene constructs with intervening P2A viral sequences to allow for equimolar expression of multiple “long” (~27 amino acids) peptides centered on the putative antigenic mutational sites. These neoantigen libraries will be cloned into an appropriate expression vector to allow for in vitro-transcription of neoantigen-encoding mRNA for use in enzyme-linked immunospot (ELISPOT) assays to detect neoantigen immune responses. RNAseq will also identify whether

common "shared" antigens are also expressed. Quantitation of mutation burden may also be important for urothelial carcinoma, where immunogenicity and/or responses to PD-1/PD-L1 axis-directed therapies may be associated with a high mutation burden.

These analyses will be performed using qualified analytes and analytic tools at the Immunopathology Lab at Fred Hutch or at a vendor, to be determined.

***Hypothesis**:* Clinical outcomes will correlate with mutational burden, and tumor-associated neoantigens will be identified that correlate with T-cell responses and clinical outcome.

11.2.1.6 *Antitumor Immune T-cell Responses – ELISPOT – Integrated Laboratory Correlative Study #6*

If there are positive clinical responses, antigen-specific T-cell responses may be assessed by IFNγ ELISPOT assay to provide information regarding the effect of the atezolizumab and NT-I7 regimen on antigen-specific T-cell functional antitumor responses such as MCC virus. The ability of the investigational treatment to induce a tumor antigen-specific immune response will be assessed using ex vivo incubation of PBMC-derived T-cells with autologous PBMC either pulsed with peptide antigens or recombinant proteins representing MCC virus or neoantigens described above. Memory viral responses (influenza A, Cytomegalovirus, Epstein-Barr Virus and Flu [CEF]) will also be assessed as controls. We will quantitate the strength of the antigen-specific T-cell responses and compare baseline and post-baseline T-cell responses for each putative antigen. The absolute change in ELISPOT responses for antigens will be compared between the spot-forming cells (SFC)/million PBMC at baseline (before treatment initiation) and at designated time points post-treatment.

Assays will be performed in the Central Immune Monitoring Laboratory (CIML) at the Fred Hutch, or an agreed upon vendor or collaborator.

***Hypothesis**:* The combination of atezolizumab with NT-I7 will result in increased T-cell responses to known and/or neoantigens.

11.2.2 *Exploratory/Ancillary Correlative Studies*

11.2.2.1 *Assessment of PD-L1 Expression at Baseline and Post-treatment -- Immunohistochemistry (IHC) – Exploratory Laboratory Correlative Study #1*

PD-L1 has been identified as an important correlative biomarker for clinical response to anti-PD-L1 and anti-PD-1 therapies. In particular, given that the function of atezolizumab is to block binding of PD-1 to PD-L1, expression of PD-L1 will be quantitated by IHC in baseline FFPE tumor specimens, and from biopsies obtained after treatment. This is an important part of the correlative studies to explore whether this marker which correlates with response in other circumstances also correlates with response in this protocol population. For baseline samples, FFPE tissue block(s) from tumor obtained before treatment will be obtained by the clinical site either from archival samples obtained from the relevant pathology laboratories where they were processed and stored, or from baseline biopsy as part of this protocol (core biopsy, punch biopsy or surgical excision). Post-treatment, FFPE tumor specimens will be obtained from biopsy at Cycle 2 (core biopsy, punch biopsy or surgical excision) as part of this protocol.

PD-L1 expression will be tested by IHC at HistoGeneX/CellCarta with the SP142 PD-L1 clone or other agreed upon vendor.

Hypothesis: PD-L1 expression at baseline will correlate with clinical responses to atezolizumab and atezolizumab in combination with NT-I7. In addition, atezolizumab administration may alter PD-L1 expression to an unknown extent.

11.2.2.2 *Evaluation of the Effect of Atezolizumab with and without NT-I7 on Circulating Lymphocyte and Monocyte Numbers and Phenotype – Exploratory Laboratory Correlative Study #2*

We will assess the effects of the atezolizumab with and without NT-I7 on the frequency and phenotypic character of PBMC subsets including dendritic cells (DCs), monocyte populations, T-cells, NK cells, and B cells. The effect on these immune cell subtypes of checkpoint inhibitors and other immune modulators is being investigated in other CITN trials and may provide important correlative information on the success or failure of combination immunotherapy.

A 21-color whole blood immunophenotyping assay will quantify in 1 panel the absolute number and proportion of T-cells (both CD8+ and CD4+), NK cells (CD56+CD3-), NKT-cells (CD56+CD3+), B cells (CD19+), monocytes (CD16+), as well as both myeloid DCs (CD45+HLA-DR+CD11c+CD123-) and plasmacytoid DCs (CD45+HLA-DR+CD11c-CD123+). In addition, to further define the phenotype and activation status of T-cells, the panel includes memory markers (CD45RA, CD197 [CCR7]), activation and Treg markers (CD28, CD127, CD25, HLA-DR, CD279 [PD-1]), and markers of exhaustion (Lag-3, Tim-3, TIGIT).

Additional whole blood immunophenotyping assays will assess the Treg population in a FoxP3 flow assay (CD45, CD3, CD4, CD25, CD127 and FoxP3) and proliferating levels of several cell subsets in a Ki67 assay (CD3, CD4, CD8, CD14, CD56 and Ki67).

For the expansion cohort, we also use a 13-color whole blood immunophenotyping assay to further quantify T cell differentiation subsets such as T_N (CD45RA+CCR7+CD95-CD127+), T_{SCM} (CD45RA+CCR7+CD95+CD127+), T_{CM} (CD45RA- CCR7+CD95+CD127+), T_{EM} (CD45RA-CCR7-CD95+CD127+/-), T_{TE} (CD45RA+CCR7-CD95+CD127-), and “Stem-like” CD8 T_{SL} (CD8+PD-1+CXCR5+TIM-3-) and exhausted T_{SL} (CD8+ PD-1+CXCR5-TIM-3+).

Assays will be performed in the CIML at the Fred Hutch.

Hypothesis: The combination of atezolizumab with NT-I7 will perturb circulating lymphocyte and monocyte numbers and phenotypes to a yet unknown extent.

11.2.2.3 *Multiplex Cytokines– Exploratory Laboratory Correlative Study #3*

Perturbations in cytokines, chemokines, and growth factors have been associated with cancers and changes in plasma cytokine concentrations of proinflammatory and immunosuppressive cytokines may correlate with clinical responses to therapy. Measurable changes in serum and plasma cytokines have been associated with checkpoint blockade therapies. Comparison of baseline plasma cytokines, chemokines and growth factors to longitudinal measurements during therapy may improve our understanding of the immunologic effects of the combination of atezolizumab plus NT-I7 on cancer. Furthermore, these assays will be used to explore whether

reactive changes in cytokine levels correlates with toxicity and/or efficacy of atezolizumab and NT-I7. Longitudinally collected plasma samples will be evaluated using commercial multiplex immunoassays (i.e., Luminex) or other appropriate technology (i.e., Olink primer extension assay) for quantitation.

Assays will be performed at the Fred Hutch Shared Resources, or at other agreed upon vendor.

Hypothesis: The combination of atezolizumab with NT-I7 will perturb cytokines, chemokines, and growth factors to a yet unknown extent.

11.2.2.4 *Kynurenine and Tryptophan (Kyn/Trp) Ratios and Plasma Arginine Levels – Exploratory Laboratory Correlative Study #4*

Over expression of IDO is known to result in increases in the kynurenine and tryptophan (Kyn/Trp) ratio in blood and within some TMEs. Increases in Kyn/Trp ratios result in the subsequent suppression of T-cell responses and is a major mechanism of immune control. IDO is expressed by some tumors, but probably more importantly, it is expressed by lymphocytes as a normal mechanism and important mechanism to dampen immune responses. Expression of IDO (with subsequent increased Kyn/Trp ratios) may be one likely mechanism of checkpoint blockade and other immunotherapy failures. Thus, we will measure Kyn/Trp ratios in plasma taken at baseline, during treatment and at end of treatment to analyze, in patients who have failed clinical response to atezolizumab plus IL-7 treatment, whether failure may be associated with increased Kyn/Trp levels.

In addition, another important mechanism of immune control and suppression is through changing concentrations of arginine (Arg) insofar as decreased levels of Arg in plasma (i.e. ARG depletion) is associated with decreases in CD4+ and CD8+ T-cell proliferation and decreased Th1 functions. Arg depletion can occur by the release by myeloid-derived suppressor cells (MDSCs) of arginase which converts L-Arg to ornithine. This may be another mechanism of tumor evasion as myeloid suppressor cells with high arginase activity are found in tumors. Thus, we will also measure Arg levels in the same plasma samples taken at baseline, during treatment and at end of treatment in this protocol to analyze, in patients who have failed clinical response to atezolizumab plus IL-7 treatment, whether failure may be associated with increased Arg levels in plasma.

All assays will be performed at an agreed upon collaborator or vendor to be determined.

Hypothesis*:* Failure to respond to atezolizumab plus NT-I7 treatment may be associated with increased Kyn/Trp ratios and/or increased plasma Arg levels in some patients.

11.2.3 *Special Studies*

11.2.3.1 *Assessment of Immunogenicity and Pharmacokinetics*

The known and predicted activities of these two immunotherapeutic agents in enhancing immune responses may, in combination, influence PK and immunogenicity. In particular, NT-I7 is pharmacologically targeting lymphocytes and subject to a PK-target mediated clearance (like many other blood growth factors). This is the case for G-CSF and other factors. We have validated methods to measure NT-I7 blood samples for PK analysis as well as to detect and

measure the presence of binding and neutralizing antibodies. We will thus measure NT-I7 blood levels before the first administration of NT-I7, at specified intervals after administration of NT-I7 and at the end of treatment visit. This would enable detection of any potential significant interference of the concomitant immunotherapies on NT-I7 PK. Similarly, we will measure anti-NT-I7 binding antibodies before the first NT-I7 administration and at specified intervals during study treatment.

Any subject presenting with binding antibodies will be further tested for the presence of anti-NT-I7 neutralizing antibodies. While the number of patients will be too small to draw definitive conclusions, this might lead to a potential interference in the combination therapy deserving a further exploration.

12. BIOMARKER, CORRELATIVE AND SPECIAL STUDIES

Specimens will be collected for several planned correlative studies. Many samples will be processed and stored to be run in batches. The CIML will provide lab kits and shipping supplies. Specific instructions on numbers and types of tubes required for each visit are provided in the ION-02 Laboratory Manual.

In the interest of patient safety, we are including a provision to draw less blood if patients are anemic. A CBC will be performed as part of the safety labs each time research labs are drawn (safety labs drawn for screening will used for this purpose prior to the first blood draws for research). The results of the CBC will be reviewed, and the following blood volumes will be drawn based upon the patient's hemoglobin level.

- Hemoglobin over 10.0 g/dL, draw the full volume of blood for safety and research labs.
- Hemoglobin between 9.0 and 10.0 g/dL, draw the full volume of blood for safety labs, CD4+ /CD8+ T-cell counts and full volume for plasma/serum-based studies. Limit research blood draws to 50 mL for cell-associated studies.
- Hemoglobin less than 9.0 g/dL, draw the full volume of blood for safety labs and CD4+ /CD8+ T-cell counts. Limit research blood draws to 5 mL for plasma and 11 mL for serum-based studies, and 30 mL for cell-associated studies.

When blood volume or specimen material is limited, the correlative studies will be considered secondary to tests needed to make clinical decisions. Prioritization of secondary tests will be made based on technical considerations: total amount of sample from each individual subject, total number of samples acquired, total number of each assay performed, and information gained from the assays performed to date. The anticipated priority correlatives are:

<u>Plasma/serum-based studies and other</u>

1. PK and immunogenicity
2. Multiplex Cytokines – Multiplex enzyme-linked immunosorbent assay (ELISA)
3. Metabolites (Kyn/Trp) ratios and Arg plasma levels

<u>Cell associated studies</u>

1. Evaluation of peripheral CD4+ and CD8+ T-cell counts
2. TCR Repertoire Analysis – TCR sequencing
3. Evaluation of the effect of atezolizumab with and without NT-I7 on circulating Lymphocyte and Monocyte numbers and Phenotypes
4. Anti-Tumor Immune T-cell Responses

Biopsy associated studies

1. Slide-Based Immune Phenotype Panel – mIHC
2. Assessment of tumor biopsy by gene expression analysis
3. Assessment of PD-L1 expression at Baseline and Post-treatment – IHC
4. Tumor-Associated Neoantigen discovery – WES and RNAseq and Antigen Prediction

12.1 Integrated Correlative Studies

12.1.1 *Evaluation of peripheral CD4+ and CD8+ T-cell counts – Integrated Laboratory Correlative Study #1*

12.1.1.1 *Collection of Specimen(s)*

Blood draws will be performed by venipuncture on study subjects in Red-Top or Gold serum-separating tubes (SSTs) or other blood draw tubes as specified per local site SOP.

12.1.1.2 *Handling of Specimens(s)*

Blood samples will be collected at room temperature and shipped ambient to the local clinical lab.

12.1.1.3 *Shipping of Specimen(s)*

Blood collection tubes will be shipped at ambient temperature to the designated local clinical, CLIA-compliant laboratory the same day as the blood draw.

12.1.1.4 *Site(s) Performing Correlative Study*

Assays will be performed at each local clinical site. Assays are to be performed at each site by a CLIA-compliant laboratory. The specific technique is not specified in the protocol.

12.1.2 *Slide-Based Immune Phenotype Panel – Multispectral IHC – Integrated Laboratory Correlative Study #2*

12.1.2.1 *Collection of Specimen(s)*

FFPE archival tissue block(s) from tumor obtained after last therapy will be identified by the clinical site at the relevant pathology laboratories where they were processed and stored. Tissue blocks are preferred, however FFPE slides can substitute if blocks are unavailable. Alternatively, patients will undergo a pre-treatment biopsy as part of this protocol. Patient will undergo a post-treatment biopsy if deemed relatively safe and technically feasible as part of this protocol.

12.1.2.2 *Handling of Specimens(s)*

FFPE archival tumor blocks will be requested by the clinical site from the relevant pathology laboratory by providing the patient's name, consent form and date of collection. Patient identifying information (i.e., name, initials, birthdate, etc.) will be removed from the specimen before shipment to the CIML. If patients undergo biopsy as part of this protocol, tissue will be placed in formalin for overnight shipping to the CIML.

12.1.2.3 *Shipping of Specimen(s)*

For archival samples, clinical sites will arrange for the FFPE tumor blocks or FFPE slides to be shipped to the CIML. For biopsies done as part of this protocol, tissue in formalin will be shipped overnight at ambient temperature to the CIML. Clinical sites will utilize BSI Engage to communicate with the CIML. After sectioning at the CIML, the CIML will coordinate shipment of unstained slides to the Immunopathology Laboratory at Fred Hutch or other agreed upon vendor or collaborator.

12.1.2.4 *Site(s) Performing Correlative Study*

Assays will be performed by the Immunopathology Lab at Fred Hutch or other agreed upon vendor or collaborator.

12.1.3 *Assessment of tumor biopsy by gene expression analysis -- Interferon γ (IFNγ) Gene Expression Signature - NanoString® nCounter® PanCancer IO 360™ Gene Expression Panel and the nCounter® PanCancer Immune Profiling Panel – Integrated Laboratory Correlative Study #3*

12.1.3.1 *Collection of Specimen(s)*

FFPE archival tissue block(s) from tumor obtained after last therapy will be identified by the clinical site at the relevant pathology laboratories where they were processed and stored. Alternatively, patients will undergo a pre-treatment biopsy as part of this protocol. Patients will undergo a post-treatment biopsy if deemed relatively safe and technically feasible as part of this protocol.

12.1.3.2 *Handling of Specimens(s)*

FFPE archival tumor blocks will be requested by the clinical site from the relevant pathology laboratory by providing the patient's name, consent form and date of collection. Patient identifying information (i.e., name, initials, birthdate, etc.) will be removed from the specimen before shipment to the CIML. If patients undergo biopsy as part of this protocol, tissue will be placed in formalin for overnight shipping to the CIML.

12.1.3.3 *Shipping of Specimen(s)*

For archival samples, clinical sites will arrange for the FFPE tumor blocks (preferred) or FFPE slides (if blocks are unavailable) to be shipped to the CIML. For biopsies done as part of this protocol, tissue in formalin will be shipped overnight at ambient temperature to the CIML. Clinical sites will utilize BSI Engage to communicate with the CIML. After sectioning at the CIML, the CIML will coordinate shipment of unstained slides (or RNA) to NanoString® or to the Immunopathology Lab at Fred Hutch. Depending on the specific contract, RNA will be extracted

from FFPE slides, either at NanoString® or the CIML, using established protocols (MAN-10050-02 Preparing Nucleic Acid from FFPE Samples for Use with nCounter® Assays).

12.1.3.4 *Site(s) Performing Correlative Study*

Assays will be performed by NanoString® Technologies in Seattle, WA, and analyzed using qualified analytic tools at the Immunopathology Lab at Fred Hutch.

12.1.4 *T-Cell Receptor Repertoire Analysis – TCR sequencing – Integrated Laboratory Correlative Study #4*

12.1.4.1 *Collection of Specimen(s)*

Specimens will include archived and prospectively obtained tumor biopsies and blood samples. Blood draws will be performed by venipuncture and whole blood will be collected into heparinized tubes.

12.1.4.2 *Handling of Specimens(s)*

Biopsy samples will be placed in formalin and shipped to the CIML. Blood collection tubes will be shipped on day of blood draw for overnight delivery to the CIML. The sample must be received at the CIML within 24 hours of blood draw. Whole blood will be submitted to Ficoll gradient centrifugation, and PBMC will be stored in aliquots of up to 20 million cells in vapor-phase liquid nitrogen.

12.1.4.3 *Shipping of Specimen(s)*

Whole blood and biopsies in formalin will be shipped overnight at ambient temperature to the CIML. Clinical sites will utilize BSI Engage to communicate with the CIML. The CIML will coordinate shipment of samples to Adaptive Biosciences or other agreed upon vendor or collaborator.

12.1.4.4 *Site(s) Performing Correlative Study*

Samples will be analyzed at Adaptive Bioscience in Seattle, WA, or other agreed upon vendor or collaborator.

12.1.5 *Tumor-Associated Neoantigen discovery – Whole Exome Sequencing and RNAseq and Antigen Prediction – Integrated Laboratory Correlative Study #5*

12.1.5.1 *Collection of Specimen(s)*

Specimens will include archived tumor blocks (or FFPE slides) and prospectively obtained tumor biopsies and blood samples. Blood draws will be performed by venipuncture.

12.1.5.2 *Handling of Specimens(s)*

For archival samples, clinical sites will arrange for the FFPE tumor specimens to be shipped to the CIML. Patient identifying information (i.e., name, initials, birthdate, etc.) will be removed from the specimen before shipment to the CIML. For biopsies done as part of this protocol, tissue in formalin will be shipped overnight at ambient temperature to the CIML where they will be embedded in paraffin. Paraffin blocks (or FFPE slides) will be stored at 4ºC under desiccant.

Blood collection tubes will be shipped on day of blood draw for overnight delivery to the CIML. Blood will be processed to PBMC as described above and stored in liquid nitrogen. Nucleic acid will be extracted from PBMC and from biopsy specimens using vendor-specific protocols.

12.1.5.3 *Shipping of Specimen(s)*

Whole blood, archival tumor blocks (or FFPE slides) and biopsies in formalin will be shipped to the CIML. Clinical sites will utilize BSI Engage to communicate with the CIML. The CIML will coordinate shipment of samples to the Immunopathology Lab at Fred Hutch and/or agreed upon vendor.

12.1.5.4 *Site(s) Performing Correlative Study*

These analyses will be performed using qualified analytes and analytic tools at the Immunopathology Lab and shared resources at Fred Hutch or at a vendor to be determined.

12.1.6 *Antitumor Immune T-cell Responses -- ELISPOT – Integrated Laboratory Correlative Study #6*

12.1.6.1 *Collection of Specimen(s)*

Blood draws will be performed by venipuncture on study subjects just before, during, and at end of treatment. Whole blood will be collected into heparinized tubes.

12.1.6.2 *Handling of Specimens(s)*

Blood collection tubes (for subsequent PBMC isolation) will be shipped on day of blood draw for overnight delivery to the CIML. The sample must be received at the CIML within 24 hours of blood draw. Whole blood will be submitted to Ficoll gradient centrifugation, and PBMC will be stored in aliquots of up to 20 million cells in vapor-phase liquid nitrogen.

12.1.6.3 *Shipping of Specimen(s)*

Blood collection tubes will be shipped overnight at ambient temperature to the CIML using CIML-established SOPs. Clinical sites will utilize BSI Engage to communicate with the CIML. The CIML will coordinate shipment via liquid nitrogen dry shipper of cryopreserved PBMC in the event that a vendor or collaborator performs these studies.

12.1.6.4 *Site(s) Performing Correlative Study*

Assays will be performed in the CIML at Fred Hutch or an agreed upon vendor or collaborator.

12.2 Exploratory Correlative Studies

12.2.1 *Assessment of PD-L1 expression at Baseline and Post-treatment -- Immunohistochemistry (IHC) – Exploratory Laboratory Correlative Study #1*

12.2.1.1 *Collection of Specimen(s)*

FFPE archival tissue block(s) from tumor obtained after last therapy will be identified by the clinical site at the relevant pathology laboratories where they were processed and stored. Tissue blocks are preferred, however FFPE slides can substitute if blocks are unavailable. Alternatively,

patients will undergo a pre-treatment biopsy as part of this protocol. Patients will undergo a post-treatment biopsy if deemed relatively safe and technically feasible as part of this protocol.

12.2.1.2 *Handling of Specimens(s)*

FFPE archival tumor blocks will be requested by the clinical site from the relevant pathology laboratory by providing the patient's name, consent form and date of collection. Patient identifying information (i.e., name, initials, birthdate, etc.) will be removed from the specimen before shipment to the CIML. If patients undergo biopsy as part of this protocol, tissue will be placed in formalin for overnight shipping to the CIML.

12.2.1.3 *Shipping of Specimen(s)*

For archival samples, clinical sites will arrange for the FFPE tumor blocks (preferred) or FFPE slides (if blocks are unavailable) to be shipped to the CIML. For biopsies done as part of this protocol, tissue in formalin will be shipped overnight at ambient temperature to the CIML. Clinical sites will utilize BSI Engage to communicate with the CIML. After sectioning at the CIML, the CIML will coordinate shipment of unstained slides to HistoGeneX/CellCarta or other agreed upon vendor.

12.2.1.4 *Site(s) Performing Correlative Study*

Assays will be performed by HistoGeneX/CellCarta or other agreed upon vendor.

12.2.2 *Evaluation of the effect of atezolizumab with and without NT-I7 on circulating Lymphocyte and Monocyte numbers and Phenotype – Exploratory Laboratory Correlative Study #2*

12.2.2.1 *Collection of Specimen(s)*

Blood draws will be performed by venipuncture on study subjects just before, during, and at end of treatment. Whole blood will be collected into heparinized tubes and ACD tubes.

12.2.2.2 *Handling of Specimens(s)*

Blood samples will be collected at room temperature and shipped ambient to the CIML the same day as the blood draw. The sample must be received at the CIML within 24 hours of blood draw. For Whole Blood immunophenotyping, aliquots of whole blood will be immediately tested using multispectral immunophenotyping panels to obtain absolute cell counts for CD4+ and CD8+ T-cell subsets and activation states, NK and NKT-cells, DC subsets, monocytes, B cells and other cell types using the 21c, 13c, and FoxP3 assays described in section 11.2.2. For Ki67 assays, blood collection tubes (for subsequent PBMC isolation) will be shipped on day of blood draw for overnight delivery to the CIML. Blood will be submitted to Ficoll gradient centrifugation, and PBMC will be stored in aliquots of up to 20 million cells in vapor-phase liquid nitrogen for storage for batch analyses.

12.2.2.3 *Shipping of Specimen(s)*

Blood collection tubes will be shipped at ambient temperature to the CIML. Clinical sites will utilize BSI Engage to communicate with the CIML.

12.2.2.4 *Site(s) Performing Correlative Study*

Flow cytometry will be performed in the CIML at Fred Hutch. Ki67 assays will be performed at the UW in collaboration with the CIML.

12.2.3 *Multiplex Cytokines -- Multiplex ELISA – Exploratory Laboratory Correlative Study #3*

12.2.3.1 *Collection of Specimen(s)*

Whole blood will be collected into red-top tubes by venipuncture.

12.2.3.2 *Handling of Specimens(s)*

Blood samples will be processed to frozen serum at the local labs and stored frozen at -80°C.

12.2.3.3 *Shipping of Specimen(s)*

Frozen serum vials will be batch-shipped quarterly from the clinical sites overnight on dry ice to the CIML. Clinical sites will utilize BSI Engage to communicate with the CIML. The CIML will coordinate shipments of serum to the Fred Hutch Shared Resource facility or other collaborator, as needed.

12.2.3.4 *Site(s) Performing Correlative Study*

Assays will be performed at the Fred Hutch Shared Resource facility or other agreed upon collaborator or vendor.

12.2.4 *Kynurenine and tryptophan (Kyn/Trp) Ratios and Plasma Arginine levels – Exploratory Laboratory Correlative Study #4*

12.2.4.1 *Collection of Specimen(s)*

Blood draws will be performed by venipuncture on study subjects at time points before, during, and after treatment. Whole blood will be collected into heparinized tubes.

12.2.4.2 *Handling of Specimens(s)*

Plasma will be isolated by the local laboratory and frozen at -80°C.

12.2.4.3 *Shipping of Specimen(s)*

Plasma samples will be batch-shipped quarterly from the clinical sites overnight on dry ice to the CIML. Sites will utilize BSI Engage to communicate with the CIML. The CIML will coordinate the shipment of samples to the agreed upon collaborator or vendor.

12.2.4.4 *Site(s) Performing Correlative Study*

Kyn/Trp and Arg levels will be assessed at an agreed upon collaborator or vendor to be determined.

12.3 Special Studies

12.3.1 *Immunogenicity Testing (NT-I7)*

The formation of ADA and neutralizing anti-drug antibodies (NADA) to NT-I7 will be evaluated at: Pre-treatment with NT-I7 (Cycle 1, Day 1) and on Day 1 of each subsequent cycle. Immunogenicity testing will be repeated at the end of treatment visit.

Patients who have positive ADA will be followed every 3 months for PD biomarkers including ALC, and serum IL-7 level until ADA level becomes undetectable.

NeoImmuneTech has transferred and revalidated the assay method to BioAgilytix for performing the assays and the determination of the main ADA/NADA parameters.

12.3.1.1 *Collection of Specimen(s)*

Whole blood will be collected in SSTs at each of the time points specified in the Study Calendar. Please refer to the ION-02 Laboratory Manual.

12.3.1.2 *Handling of Specimen(s)*

Blood samples will be handled as per the draw tube manufacturer's recommendations, processed at the local labs into serum aliquots, and aliquots stored frozen at -80°C until shipped to the CIML.

12.3.1.3 *Shipping of Specimen(s)*

Frozen samples will be batch-shipped quarterly, or immediately upon collection of EOT visit from the clinical sites overnight on dry ice to the CIML. Sites will utilize BSI Engage to communicate with the CIML. The CIML will coordinate the shipment of samples to BioAgilytix.

12.3.1.4 *Site(s) Performing Correlative Study*

Assays will be performed by BioAgilytix, Durham, NC.

12.3.2 *NT-I7 Pharmacokinetics*

PK samples will be collected on all patients (dose escalation and dose expansion phase). Serial samples will be assessed following the PK timepoints in the tables below.

Note: The precise NT-I7 administration time and the precise PK draw time should be recorded accurately and prospectively to fully interpret these values. Blood draws are timed after NT-I7 administration is complete, not completion of atezolizumab infusion.

12.3.2.1 *Collection of Specimen(s)*

Blood draws will be performed by venipuncture on study subjects before, during, and at end of treatment.

Table 5 – Pharmacokinetic Timepoints

Dose Escalation (Phase 1b) – all patients

Pharmacokinetics										
Treatment Cycle/Visit	**Cycle 1**					**Cycle 3**		**Cycle 5**		**End of Tx**
Scheduling Window	**Day 1**			**Day 2***	**Day 8 ± 2**	**Day 1**		**Day 1**		**30 days ± 5**
Hours	0	2 (±5mins)	6 (±15mins)	24 (±30mins)		0	2 (±5mins)	0	2 (±5mins)	
PK	X[a]	X[b]	X[b]	X	X	X[a]	X[b]	X[a]	X[b]	X

a To be collected prior to study agent(s) administration on a dosing day

b Blood draws are timed after NT-I7 administration is complete, not completion of atezolizumab infusion.

* Day 2 is an optional blood draw

Dose Expansion (Phase 2a) – all patients

Pharmacokinetics								
Treatment Cycle/Visit	**Cycle 1**			**Cycle 3**		**Cycle 5**		**End of Tx**
Scheduling Window	**Day 1**		**Day 8 ± 2**	**Day 1**		**Day 1**		**30 days ± 5**
Hours	0	2 (±5mins)		0	2 (±5mins)	0	2 (±5mins)	
PK	X[a]	X[b]	X	X[a]	X	X[a]	X	X

a To be collected prior to study agent(s) administration on a dosing day

b Blood draws are timed after NT-I7 administration is complete, not completion of atezolizumab infusion.

12.3.2.2 *Handling of Specimen(s)*

Serum will be isolated by the local laboratory and frozen at -80°C until shipped to the CIML.

12.3.2.3 *Shipping of Specimen(s)*

Frozen samples will be batch-shipped quarterly from the clinical sites overnight on dry ice to the CIML. Sites will utilize BSI Engage to communicate with the CIML. The CIML will coordinate the shipment of samples to the agreed upon collaborator or vendor.

12.3.2.4 *Site(s) Performing Correlative Study*

Assays will be performed by the agreed upon collaborator or vendor to be determined.

13. DATA REPORTING / REGULATORY REQUIREMENTS / STUDY OVERSIGHT

13.1 Data Reporting

Data collection for this study will be done exclusively through an electronic clinical data management system. The Data Management Organization will utilize a core set of electronic Case Report Forms (eCRF) that are Clinical Data Acquisition Standards Harmonization (CDASH)-compliant (https://www.cdisc.org/standards/foundational/cdash). Customized eCRFs will be included when appropriate to meet unique study requirements. The data management organization will prepare the eCRFs with built-in edit checks to the extent possible to promote data integrity.

Access to the trial is granted to all persons with the appropriate roles, as listed on the Delegation of Duties Sheet. Upon site activation, all persons with the appropriate roles will be sent a study invitation email. Site users will not be able to access the study until all required study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings).

For further details, please refer to the Study Procedures Manual.

13.1.1 *Responsibility for Data Submission*

It is the responsibility of the PI(s) at the site to ensure that all investigators and study staff at the clinical site understand the procedures for data submission per the protocol. Furthermore, protocol-specified data must be submitted accurately and in a timely manner to the data management organization via the EDC system.

The data management organization, in collaboration with the ION Coordinating Center, is responsible for compiling and submitting data to the study sponsor for all participants and for providing the data to the Protocol PI for review.

13.2 Compliance with Trial Registration and Results Posting Requirements

Under the terms of the Food and Drug Administration Modernization Act (FDAMA) and the Food and Drug Administration Amendments Act (FDAAA), the study sponsor is solely responsible for determining whether the trial and its results are subject to the requirements for submission to the Clinical Trials Data Bank, https://www.clinicaltrials.gov. Information posted will allow patients to identify potentially appropriate trials for their disease conditions and pursue participation by calling a central contact number for further information on appropriate trial locations and trial site contact information.

13.3 Study Oversight

This protocol will adhere to the policies and requirements listed in Appendix B. The specific responsibilities of the PI, the ION Coordinating Center and NeoImmuneTech and the procedures for auditing are presented in Appendix B.

- The contract research organization (CRO), IQVIA, is responsible for distributing all Investigational New Drug Application (IND) Action Letters or Safety Reports received from

the study sponsor, NeoImmuneTech, to all participating institutions for submission to their individual Institutional Review Boards (IRBs) for action as required.

- Investigational agents may be ordered by a participating site only after approved by the study sponsor, NeoImmuneTech, and officially activated by the ION Coordinating Center.

14. SITE ACTIVATION PROCEDURES

14.1 IRB Approval

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit all required regulatory documents (including any protocol-specific documents) to the CRO, IQVIA, before they can be approved to enroll patients. IQVIA uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to:

- An active Federal Wide Assurance (FWA) number
- A complete Delegation of Duties Sheet (which must be maintained and kept current)
- A valid IRB approval
- Compliance with all protocol-specific requirements

In addition, the site-protocol PI must meet the following criteria:

- Complete Form FDA 1572
- Complete Financial Disclosure
- Current Curriculum Vitae
- Valid Medical License

Evidence of the following training must be provided for the site-protocol PI, sub-investigators and all participating clinical site staff, as reported on the Form FDA 1572 and Delegation of Duties Sheet:

- Current GCP Training
- Current Human Subject Protection (HSP) Training
- Evidence of Protocol/Amendments Training

14.1.1 *Submitting Regulatory Documents*

Submit your IRB approval, model Informed Consent Form (ICF), and other protocol-specific regulatory documentation to IQVIA, where they will be filed and tracked.

Regulatory Submission of Essential Documents (pre-site activation) to IQVIA by e-mail to NIT106EDP@iqvia.com.

14.2 Patient Enrollment

Patient enrollment will be facilitated using the enrollment module in the EDC system. To access the enrollment module, the site user must be assigned the 'Enroll/Randomize participants' responsibility on the ION Delegation of Duties Sheet.

Before enrolling a patient in the EDC system, registration staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes.
- If applicable, all patients have signed an appropriate consent form and Health Insurance Portability and Accountability Act of 1996 (HIPAA) authorization form (if applicable).

For further instructional information on patient enrollment; please refer to the Study Procedures Manual or contact the ION Coordinating Center at ion@fredhutch.org.

14.3 General Guidelines

After enrollment, patients should begin protocol treatment within 3 business days. Issues that would cause treatment delays should be discussed with the PI. If a patient does not receive protocol therapy after enrollment, the patient's enrollment on the study may be canceled. The Clinical Research Site (CRS) must notify the ION Coordinating Center of cancellations as soon as possible.

15. STATISTICAL CONSIDERATIONS

This section outlines the statistical analysis strategy and methods for the study. If, after the study has begun, but before the conduct of any analysis, changes are made to primary and/or key secondary hypotheses or the statistical methods related to those hypotheses, then the protocol will be amended (consistent with ICH Guideline E-9). Changes to exploratory or other non-confirmatory analyses made after the protocol has been finalized, along with an explanation as to when and why they occurred, will be listed in the Clinical Study Report (CSR) for the study. Post hoc exploratory analyses will be clearly identified in the CSR. No separate Statistical Analysis Plan (SAP) will be issued for this study.

15.1 Responsibility for Analyses

The statistical analysis of the data obtained from this study will be the responsibility of Fred Hutch as part of the ION.

15.2 Study Design/Objectives

The proposed clinical trial is a phase 1b/2a, open-label, multicenter study of the administration of atezolizumab (MPDL3280A) in combination with NT-I7 in patients with anti-PD-1/PD-L1 naïve or relapsed/refractory high-risk skin cancers: cSCC, MCC and melanoma.

This study will evaluate the safety and anti-tumor activity of NT-I7 in combination with atezolizumab, including estimation of the MTD and/or the RP2D. The trial is designed to

determine whether NT-I7, at doses known to increase the peripheral blood T-cell level, will also increase the level of tumor infiltrating T-cells and thereby increases the efficacy of atezolizumab.

15.2.1 *Dose Escalation Phase*

A 3+3 dose escalation design will be utilized. Four pre-specified doses of NT-I7 (120, 360, 840, and 1200 µg/kg) will be tested (refer to Section 5). The initial dose level will start at 120 µg/kg. NT-I7 doses will be escalated according to the dose escalation scheme in Section 5.2.2, 1200 µg/kg as the MAD. Enrollment will continue until the MTD or RP2D is identified, or up to the MAD.

The RP2D will be selected according to the following logic, taking into account the MTD determination from the Dose Escalation Phase (Phase 1b) and the MED level which is defined as the dose level at which maximum effects on peripheral blood T-cell levels and intratumor T-cell levels are observed. The intratumor T-cell levels will dominate if the peripheral blood and intratumor T-cell levels differ. The available data will be assessed by the Data and Safety Monitoring Committee, which includes the ION Coordinating Center, the Protocol PI, clinical sites PIs and NeoImmuneTech's Chief Medical Officer or designee to select the RP2D.

- If the MTD is determined AND
    - MTD = MED, then the RP2D = MTD = MED
    - MTD > MED, then the RP2D = MED
- If the MTD is not reached, then the RP2D = MED
- Once the RP2D has been selected, the trial will proceed to the Dose Expansion Phase (Phase 2a) to further evaluate RP2D in a larger number of patients.

15.2.2 *Expansion Phase*

The selected RP2D will be further evaluated in the Phase 2a.

The Phase 2a is designed to evaluate the anti-tumor effects of NT-I7 in combination with atezolizumab in a total of 60 patients. Twenty-four patients in Arm I, i.e., 12 patients for each indication (cSCC and MCC), and 36 patients in Arm II, i.e., 12 patients for each indication (MCC, cSCC and melanoma), will be enrolled. Enrolled patients will be separated into 2 arms depending on previous use of anti-PD-1/PDL-1 therapy and cancer type (see Section 5).

15.3 Sample Size/Accrual Rate

During the dose escalation phase (Phase 1b), a 3+3 design will be used for identifying the MTD and/or the RP2D. The following design will be used:

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enroll 3 patients at the next dose level.
1 out of 3	Enroll at least 3 more patients at this dose level. • If 0 of these 3 additional patients experience a DLT (totaling 1/6), escalate to the next dose level. • If ≥1 of these 3 additional patients experiences DLT (totaling ≥2/6 at the dose level), the dose escalation is stopped, and the next lower dose level will be declared the MTD. In the case this happens at the 120 µg/kg dose level, a lower dose of 60 µg/kg will be tested, and the same 3+ 3 design followed.
≥2 out of 3	Dose escalation will be stopped. The next lower dose level will be declared the MTD. Three (3) additional patients will be entered at the next lower dose level if only 3 patients were treated previously at that dose. In the case this happens at the 120 µg/kg dose level, a lower dose of 60 µg/kg will be tested, and the same 3+3 design followed.

Abbreviations: DLT=dose-limiting toxicity; MTD=maximum tolerated dose.

Once all patients in a cohort have completed the 3-week DLT window, all AEs will be assessed by the Data and Safety Monitoring Committee.

In the dose expansion phase (Phase 2a), a total of 60 patients will be treated at the RP2D as defined above.

Based on the available data provided in Section 2.2, the average historical ORR for the patients in this study would be 45.25% in Arm I and less than 5% in Arm II. With a total of 24 patients treated at the RP2D in Phase 2a of the study in Arm I, the 95% confidence interval for an observed ORR of 17/24 or higher would exclude 45.25%. With a total of 36 patients treated at the RP2D in Phase 2a of the study in Arm II, the 95% confidence interval for an observed ORR of 6/36 or higher would exclude 5%.

Approximately 84 patients will be enrolled in this study; up to approximately 24 patients in the Phase 1b and approximately 60 evaluable patients in the Phase 2a of the study. The expected accrual is 4 patients per month total at approximately 8 ION sites.

Safety Population: All patients who receive at least 1 dose of the investigational regimen will be considered evaluable for safety and included in the overall safety analysis. For the Phase 1b, in the absence of a DLT, patients must complete the full 3-week DLT window period to be considered evaluable for DLTs. Patients who discontinue from the study before the completion of the full 3-week (21 days) DLT window for reasons other than the occurrence of a DLT (e.g., withdrawal of consent, rapid tumor progression, death due to rapid tumor progression, AE that does not meet DLT criteria) will not be considered evaluable for DLTs and will be replaced.

Efficacy Population: The efficacy analysis for the Phase 1b (dose escalation) portion will be conducted on all DLT-evaluable patients, as defined in Section 6.2.2. The efficacy analysis for the Phase 2a (expansion phase) portion will be conducted on all patients who received at least one cycle of study treatment and have at least one evaluable post baseline tumor assessments.

15.4 Stratification Factors: N/A

15.5 Analysis of Secondary Endpoints

15.5.1 *Secondary Objectives*

Statistical analyses will be conducted for the following secondary objectives:

- The immunogenicity of NT-I7
- A preliminary assessment of the anti-tumor activity of NT-I7 in combination with atezolizumab, including the ORR, as measured by RECIST v1.1 and iRECIST, the DCR, DOR, PFS, and OS.

Descriptive statistics will be provided. Mean, standard deviation, median, minimum and maximum will be displayed for continuous variables, and numbers and percentages will be displayed for counts and binary variables. Kaplan-Meier estimates will be considered for time to event variables (DOR, PFS and OS). The analyses will be performed for each arm in Phase 2a, separately.

15.5.2 *Exploratory Endpoints*

Statistical analyses will be conducted to determine the immune correlates of the clinical activity of the investigational treatment combination.

The endpoints will include the evaluation of the effect of the investigational treatment combination on the immune-bias of the TME, based upon baseline and post-baseline tumor biopsy comparisons of:

- Serum concentration of NT-I7 administered in combination with atezolizumab at specified timepoints for the following parameters: Area under the concentration time-curve (AUC), maximum serum concentration (C_{max}), minimum serum concentration (C_{min}), Clearance (CL)
- Number, distribution, and phenotype of tumor-infiltrating cells
- PD-L1 expression
- Expression of IFNγ and associated proinflammatory gene expression in the TME
- Changes in TME that correlate with response or provide information on potential actionable causes for lack of clinical benefit.

15.6 Reporting and Exclusions

15.6.1 *Evaluation of Toxicity*

All patients will be evaluable for toxicity from the time of their first treatment with atezolizumab and/or NT-I7 until 30 days after the last dose of study treatment.

15.6.2 *Evaluation of Response*

All patients enrolled in the study who received at least 1 cycle of study treatment will be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: (1) CR, (2) PR, (3) SD, (4) PD, (5) early death from malignant disease, (6) early death from toxicity, (7) early death because of other cause, or (9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

All of the patients who met the eligibility criteria and received at least 1 dose of study treatment will be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on patients who received at least 1 cycle of study treatment. Sub-analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (early death due to other reasons, early discontinuation of treatment, major protocol violations, *etc.*). However, these sub-analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

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APPENDIX A PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

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 (CIOMS) International Ethical Guidelines.
  - Applicable ICH Good Clinical Practice (GCP) Guidelines.
  - Applicable laws and regulations.
- The protocol, protocol amendments, ICF, Investigator's Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB/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 and regulatory authority approval, when applicable, before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to subjects.
- 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 SAEs or other significant safety findings as required by IRB/IEC procedures.
  - Providing oversight of the conduct of the study at the study center and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations.
- After reading the protocol, each Investigator will sign the protocol signature page and send a copy of the signed page to IQVIA by e-mail to a contact [redacted] (Appendix D). The study will not start at any study center at which the Investigator has not signed the protocol.

Adequate Resources

The Investigator is responsible for supervising any individual or party to whom the Investigator delegates study-related duties and functions conducted at the study center.

If the Investigator/institution retains the services of any individual or party to perform study related duties and functions, the Investigator/institution should ensure this individual or party is qualified to perform those study-related duties and functions and should implement procedures to ensure the integrity of the study-related duties and functions performed and any data generated.

Financial Disclosure

Investigators and sub-Investigators will provide the Sponsor, via submission to IQVIA, 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.

Informed Consent Process

- The Investigator or his/her representative will explain the nature of the study to the subject and answer all questions regarding the study.
- Subjects must be informed that their participation is voluntary. Subjects will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the subject was entered in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the subject.

Data Protection

- Subjects will be assigned a unique identifier. Any subject records or datasets that are transferred to the Sponsor will contain the identifier only; subject names or any information which would make the subject identifiable will not be transferred.
- The subject 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 subject.
- The subject 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 ICF will incorporate (or, in some cases, be accompanied by a separate document incorporating) wording that complies with relevant data protection and privacy legislation.
- The Sponsor or its representative will not provide individual genotype results to subjects, any insurance company, any employer, their family members, general physician, or any other third party, unless required to do so by law.
- Extra precautions are taken to preserve confidentiality and prevent genetic data being linked to the identity of the subject. In exceptional circumstances, however, certain individuals might see both the genetic data and the personal identifiers of a subject. For example, in the case of a medical emergency, the ION Coordinating Center, Sponsor or representative physician or an Investigator might know a subject's identity and have access to his or her genetic data. Also, regulatory authorities may require access to the

relevant files.

Administrative Structure

The Data and Safety Monitoring committee will include, at a minimum, NeoImmuneTech's Chief Medical Officer or designee, the Protocol PI, clinical site PIs and the ION Coordinating Center. The Principal Investigator and NeoImmuneTech, when appropriate, will invite other specialist individuals to participate in the review, e.g., PK scientists, statisticians, clinical specialists etc.

Table 6 – Study Administrative Structure

Function	**Responsible Organization**
Study Operations Management	ION Coordinating Center
Medical Monitoring	ION Coordinating Center and Protocol PI
On-site clinical monitoring	CRO – Avance Clinical, LLC
Study Master File	CRO – IQVIA
Data Management	CRO – Cytel, Inc.
Clinical Supply Management	Drug Information System integrated to eDC system
Biostatistics	CRO – Cytel, Inc.
Medical Writing	ION Coordinating Center and CRO - IQVIA
Data and Safety Monitoring Committee	Protocol PI, clinical site PIs, ION Coordinating Center and NIT's Chief Medical Officer or designee

Dissemination of Clinical Study Data

The results of the study should be reported within 1 year from the end of the clinical study. Irrespective of the outcome, the Sponsor, NeoImmuneTech, will submit to the Food and Drug Administration a summary of the results of the clinical study within 1 year from the end of the clinical study. It shall be accompanied by a summary written in a manner that is understandable to laypersons.

Data Quality Assurance

- All subject data relating to the study will be transmitted electronically. The Investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.
- 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.
- The ION Coordinating Center or designee is responsible for the data management of this study including quality checking of the data.
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized study center personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects 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.
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator for 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after the investigation is discontinued and FDA is notified. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

Source Documents

The Investigator/institution should maintain adequate and accurate source documents and study records that include all pertinent observations on each of the study center's subjects. Source data should be attributable, legible, contemporaneous, original, accurate, and complete. Changes to source data should be traceable, should not obscure the original entry, and should be explained if necessary (e.g., via an audit trail).

- Source documents provide evidence for the existence of the subject and substantiate the integrity of the data collected. Source documents are filed at the Investigator's study center.
- Data entered in the eCRF 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.
- Definition of what constitutes source data can be found in the ION Study Procedures Manual.

Study and Study Center Closure

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

The Investigator may initiate study center 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 center by the Sponsor, NeoImmuneTech or the ION Coordinating Center 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 subjects by the Investigator.
- Discontinuation of further study treatment development.

Publication Policy

The data generated by this study are confidential information of the Sponsor. The Sponsor will make the results of the study publicly available. The publication policy with respect to the Investigator and study center will be set forth in the Clinical Trial Agreement.

- 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, NeoImmuneTech, before submission. This allows NeoImmuneTech 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, NeoImmuneTech will generally support publication of multicenter studies only in their entirety and not as individual study center data. In this case, one of the ION Investigators will be designated, by mutual agreement, as the Coordinating Investigator.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

APPENDIX C NT-I7 DILUTION AND IM INJECTION GUIDELINES

NT-I7 will be injected by intramuscular route to be beneficial for absorption and injection site reaction.

For the best possible injection accuracy and injection volume consistency in a lower dose, i.e., if the number of patients experiencing DLT at Dose Level (DL) 1, 120 µg/kg, requires dose reduction to 60 µg/kg (Section 5.2), NT-I7 will be diluted by adding the appropriate amount of NT-I7 into NT-I7 Diluent. At the dose level of 60 µg/kg, diluted NT-I7 at a concentration of 12.5 mg/mL will be used by adding NT-I7 (25 mg/mL) 1 mL to Diluent 1 mL*.

For Dose Level (DL) 1, 2, 3 and 4, NT-I7 25 mg/mL will be used without dilution.

Dose volumes for each dose level is based on the patient's actual body weight. Body weight should be rounded to the nearest whole kilogram. For obese patients (BMI $\geq$ 30 kg/m^2), refer to instructions in Section 6.1.1 for calculating the Adjusted Body Weight before referring to the chart below.

When IM injections are administered, maximum volumes have been proposed across the various IM sites with 0.5-2 mL and the recommended volume for the deltoid site is up to 1 mL because the mid-deltoid site has a small muscle mass. The proposed method is able to meet the injection volume to less than 2 mL across all dose levels which may minimize both patient's discomfort related to IM injection and variability of actual dispensing amount given the leftover in the needle and syringe. Dose volumes are shown in the Dose and Injection Volume Chart below.

In addition, at higher dose levels, the intramuscular injection volume $\geq$ 1mL may cause injection site pain. Therefore, dose volumes greater than 1 mL may be divided into 2 injections and the maximum volume that may be administered per intramuscular injection is 1 mL (refer to Section 6.1.1).

Dose and Injection Volume Chart

Body weight (kg)	120 µg/kg (DL 1)			360 µg/kg (DL 2)			840 µg/kg (DL 3)			1200 µg/kg (DL 4)		
	25 mg/mL			25 mg/mL			25 mg/mL			25 mg/mL		
	dose (µg)	volume (mL)	# Injections	dose (µg)	volume (mL)	# Injections	dose (µg)	volume (mL)	# Injections	dose (µg)	volume (mL)	# Injections
50	6000	0.24	1	18000	0.72	1	42000	1.68	2	60000	2.40	3
51	6120	0.24	1	18360	0.73	1	42840	1.71	2	61200	2.45	3
52	6240	0.25	1	18720	0.75	1	43680	1.75	2	62400	2.50	3
53	6360	0.25	1	19080	0.76	1	44520	1.78	2	63600	2.54	3
54	6480	0.26	1	19440	0.78	1	45360	1.81	2	64800	2.59	3
55	6600	0.26	1	19800	0.79	1	46200	1.85	2	66000	2.64	3
56	6720	0.27	1	20160	0.81	1	47040	1.88	2	67200	2.69	3
57	6840	0.27	1	20520	0.82	1	47880	1.92	2	68400	2.74	3
58	6960	0.28	1	20880	0.84	1	48720	1.95	2	69600	2.78	3
59	7080	0.28	1	21240	0.85	1	49560	1.98	2	70800	2.83	3

Body weight (kg)	120 µg/kg (DL 1)			360 µg/kg (DL 2)			840 µg/kg (DL 3)			1200 µg/kg (DL 4)		
	25 mg/mL			25 mg/mL			25 mg/mL			25 mg/mL		
	dose (µg)	volume (mL)	# Injections	dose (µg)	volume (mL)	# Injections	dose (µg)	volume (mL)	# Injections	dose (µg)	volume (mL)	# Injections
60	7200	0.29	1	21600	0.86	1	50400	2.02	3	72000	2.88	3
61	7320	0.29	1	21960	0.88	1	51240	2.05	3	73200	2.93	3
62	7440	0.30	1	22320	0.89	1	52080	2.08	3	74400	2.98	3
63	7560	0.30	1	22680	0.91	1	52920	2.12	3	75600	3.02	4
64	7680	0.31	1	23040	0.92	1	53760	2.15	3	76800	3.07	4
65	7800	0.31	1	23400	0.94	1	54600	2.18	3	78000	3.12	4
66	7920	0.32	1	23760	0.95	1	55440	2.22	3	79200	3.17	4
67	8040	0.32	1	24120	0.96	1	56280	2.25	3	80400	3.22	4
68	8160	0.33	1	24480	0.98	1	57120	2.28	3	81600	3.26	4
69	8280	0.33	1	24840	0.99	1	57960	2.32	3	82800	3.31	4
70	8400	0.34	1	25200	1.01	2	58800	2.35	3	84000	3.36	4
71	8520	0.34	1	25560	1.02	2	59640	2.39	3	85200	3.41	4
72	8640	0.35	1	25920	1.04	2	60480	2.42	3	86400	3.46	4
73	8760	0.35	1	26280	1.05	2	61320	2.45	3	87600	3.50	4
74	8880	0.36	1	26640	1.07	2	62160	2.49	3	88800	3.55	4
75	9000	0.36	1	27000	1.08	2	63000	2.52	3	90000	3.60	4
76	9120	0.36	1	27360	1.09	2	63840	2.55	3	91200	3.65	4
77	9240	0.37	1	27720	1.11	2	64680	2.59	3	92400	3.70	4
78	9360	0.37	1	28080	1.12	2	65520	2.62	3	93600	3.74	4
79	9480	0.38	1	28440	1.14	2	66360	2.65	3	94800	3.79	4
80	9600	0.38	1	28800	1.15	2	67200	2.69	3	96000	3.84	4
81	9720	0.39	1	29160	1.17	2	68040	2.72	3	97200	3.89	4
82	9840	0.39	1	29520	1.18	2	68880	2.76	3	98400	3.94	4
83	9960	0.40	1	29880	1.20	2	69720	2.79	3	99600	3.98	4
84	10080	0.40	1	30240	1.21	2	70560	2.82	3	100800	4.03	5
85	10200	0.41	1	30600	1.22	2	71400	2.86	3	102000	4.08	5
86	10320	0.41	1	30960	1.24	2	72240	2.89	3	103200	4.13	5
87	10440	0.42	1	31320	1.25	2	73080	2.92	3	104400	4.18	5
88	10560	0.42	1	31680	1.27	2	73920	2.96	3	105600	4.22	5
89	10680	0.43	1	32040	1.28	2	74760	2.99	3	106800	4.27	5
90	10800	0.43	1	32400	1.30	2	75600	3.02	4	108000	4.32	5
91	10920	0.44	1	32760	1.31	2	76440	3.06	4	109200	4.37	5
92	11040	0.44	1	33120	1.32	2	77280	3.09	4	110400	4.42	5
93	11160	0.45	1	33480	1.34	2	78120	3.12	4	111600	4.46	5
94	11280	0.45	1	33840	1.35	2	78960	3.16	4	112800	4.51	5
95	11400	0.46	1	34200	1.37	2	79800	3.19	4	114000	4.56	5
96	11520	0.46	1	34560	1.38	2	80640	3.23	4	115200	4.61	5
97	11640	0.47	1	34920	1.40	2	81480	3.26	4	116400	4.66	5

Body weight (kg)	120 µg/kg (DL 1)			360 µg/kg (DL 2)			840 µg/kg (DL 3)			1200 µg/kg (DL 4)		
	25 mg/mL			25 mg/mL			25 mg/mL			25 mg/mL		
	dose (µg)	volume (mL)	# Injections	dose (µg)	volume (mL)	# Injections	dose (µg)	volume (mL)	# Injections	dose (µg)	volume (mL)	# Injections
98	11760	0.47	1	35280	1.41	2	82320	3.29	4	117600	4.70	5
99	11880	0.48	1	35640	1.43	2	83160	3.33	4	118800	4.75	5
100	12000	0.48	1	36000	1.44	2	84000	3.36	4	120000	4.80	5
101	12120	0.48	1	36360	1.45	2	84840	3.39	4	121200	4.85	5
102	12240	0.49	1	36720	1.47	2	85680	3.43	4	122400	4.90	5
103	12360	0.49	1	37080	1.48	2	86520	3.46	4	123600	4.94	5
104	12480	0.50	1	37440	1.50	2	87360	3.49	4	124800	4.99	5
105	12600	0.50	1	37800	1.51	2	88200	3.53	4	126000	5.04	6
106	12720	0.51	1	38160	1.53	2	89040	3.56	4	127200	5.09	6
107	12840	0.51	1	38520	1.54	2	89880	3.60	4	128400	5.14	6
108	12960	0.52	1	38880	1.56	2	90720	3.63	4	129600	5.18	6
109	13080	0.52	1	39240	1.57	2	91560	3.66	4	130800	5.23	6
110	13200	0.53	1	39600	1.58	2	92400	3.70	4	132000	5.28	6
111	13320	0.53	1	39960	1.60	2	93240	3.73	4	133200	5.33	6
112	13440	0.54	1	40320	1.61	2	94080	3.76	4	134400	5.38	6
113	13560	0.54	1	40680	1.63	2	94920	3.80	4	135600	5.42	6
114	13680	0.55	1	41040	1.64	2	95760	3.83	4	136800	5.47	6
115	13800	0.55	1	41400	1.66	2	96600	3.86	4	138000	5.52	6
116	13920	0.56	1	41760	1.67	2	97440	3.90	4	139200	5.57	6
117	14040	0.56	1	42120	1.68	2	98280	3.93	4	140400	5.62	6
118	14160	0.57	1	42480	1.70	2	99120	3.96	4	141600	5.66	6
119	14280	0.57	1	42840	1.71	2	99960	4.00	4	142800	5.71	6
120	14400	0.58	1	43200	1.73	2	100800	4.03	5	144000	5.76	6

Guidelines for Injecting NT-I7 Solution by Research Nurse or Investigator:

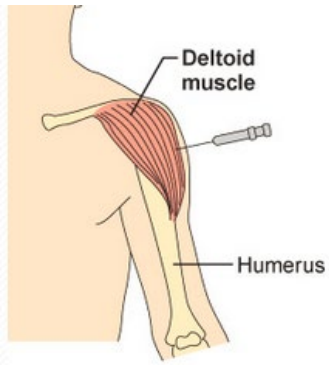
- With one hand, gently press the cleaned area of skin and hold it firmly. With the other hand, hold the syringe at about a 90º angle to the skin.
- With a quick, short motion, push the needle through the skin into the muscle.
- With your free hand, slowly push the plunger to inject solution.
- When the syringe is empty, remove the needle from the skin being careful to keep it at the same angle it was when it was inserted.

Press a cotton ball over the injection site for 10 seconds. Slight bleeding may occur. **DO NOT** rub the injection site. A bandage is optional.

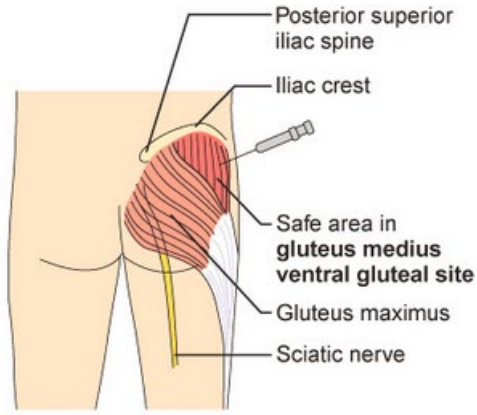
Injection site

NT-I7 will be injected by intramuscular route. The best areas for injection are the deltoid muscle and the muscles of the thighs and buttock; it should remain away from joints, nerves, bones, and other important structures.

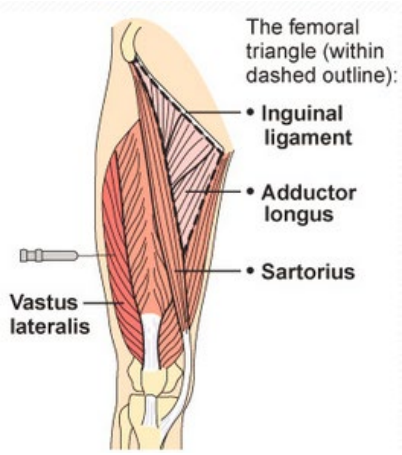
Do not use the same site for injection when the volume of injection requires several injections to a patient. Use the attached Injection Site Diary and the site codes below to document the injection site(s).



Left Deltoid ‘A’ Right Deltoid ‘B’



Left Buttock ‘C’ Right Buttock ‘D’



Left Thigh ‘E’ Right Thigh’F’

INJECTION SITE DIARY

To be completed by Research Nurse or Investigator

Patient Name _____ **Patient Study ID** _____

Injection	**Date** (MM/DD/YYYY)	**Injection Site**
#1		❒ A ❒ B ❒ C ❒ D ❒ E ❒ F
#2		❒ A ❒ B ❒ C ❒ D ❒ E ❒ F
#3		❒ A ❒ B ❒ C ❒ D ❒ E ❒ F
#4		❒ A ❒ B ❒ C ❒ D ❒ E ❒ F
#5		❒ A ❒ B ❒ C ❒ D ❒ E ❒ F
#6		❒ A ❒ B ❒ C ❒ D ❒ E ❒ F
#7		❒ A ❒ B ❒ C ❒ D ❒ E ❒ F

APPENDIX D SIGNATURE OF INVESTIGATOR

PROTOCOL TITLE: A phase 1b/2a, open label study to evaluate anti-tumor efficacy and safety of rhIL-7-hyFc (NT-I7) in combination with anti-PD-L1 (atezolizumab) in patients with anti-PD-1/PD-L1 naïve or relapsed/refractory high-risk skin cancers

PROTOCOL NUMBER: NIT-106 (ION-02)

VERSION: Amendment 5, v1.0, dated January 19, 2023

This protocol is a confidential communication of NeoImmuneTech. I confirm that I have read this protocol, I understand it, and I will work according to this protocol. I will also work consistently with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with Good Clinical Practice and the applicable laws and regulations. Acceptance of this document constitutes my agreement that no unpublished information contained herein will be published or disclosed without prior written approval from the Sponsor.

Instructions to the Investigator: Please SIGN and DATE this signature page. PRINT your name, title, and the name of the study center in which the study will be conducted. Return the signed copy to IQVIA by e-mail to [redacted]

I have read this protocol in its entirety and agree to conduct the study accordingly:

Signature of Investigator: _____ Date: _____

Printed Name: _____

Investigator Title: _____

Name/Address of Center: _____

_____

_____

_____

APPENDIX E ABBREVIATIONS LIST

ADA	anti-drug antibodies
AE	adverse event
ALC	absolute lymphocyte count
ALT	alanine aminotransferase
AML	acute myelocytic leukemia
ANC	absolute neutrophil count
Anti-HBc	antibody to hepatitis B core antigen
Anti-TNF	antitumor necrosis factor
aPTT	activated partial thromboplastin time
Arg	arginine
AST	aspartate aminotransferase
AUC	area under the concentration time-curve
BAL	bronchoscopic alveolar lavage
BSA	body surface area
BUN	blood urea nitrogen
CAP	chest, abdomen, and pelvis
CBC	complete blood count
CDASH	Clinical Data Acquisition Standards Harmonization
CHO	Chinese Hamster Ovary
CIML	Central Immune Monitoring Laboratory
CITN	Cancer Immunotherapy Trials Network
CL	clearance
CLIA	Clinical Laboratory Improvement Amendments
CM	central memory
C_{max}	maximum serum concentration
C_{min}	minimum serum concentration
CNS	central nervous system
COPD	chronic obstructive pulmonary disease
CPI	checkpoint Inhibitor
CR	complete response
CRF	case report form

CRO	contract research organization
CRS	clinical research site
cSCC	cutaneous squamous cell carcinoma
CSR	clinical study report
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DC	dendritic cell
DCR	disease control rate
DLT	dose-limiting toxicity
DOR	duration of objective response
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
EDC	electronic data capture
EKG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot
EM	effector memory
EOT	end of treatment
EU GMP	European Union – Good Manufacturing Practice
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act
FDAMA	Food and Drug Administration Modernization Act
FDG	fluorodeoxyglucose
FFPE	formalin-fixed paraffin-embedded
FIH	first-in-human
FU	follow-up
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
G-CSF	granulocyte-colony stimulating factor
GEP	gene expression programming
HAART	highly active antiretroviral therapy
HBsAg	hepatitis B surface antigen

HBV	hepatitis B virus
HCV	hepatitis C virus
HED	human equivalent dose
HIPAA	Health Insurance Portability and Accountability Act of 1996
HIV	human immunodeficiency virus
HSP	Human Subject Protection
hyFC	hybrid Fc
IB	Investigator's Brochure
IC	immune cells
ICF	informed consent form
ICH	International Council for Harmonisation
IDO	indoleamine 2,3-dioxygenase
IFN-γ(-α, etc)	interferon-gamma (alpha, etc)
IgD	Immunoglobulin D
IgG(1,2…)	immunoglobulin G (1,2…)
IHC	immunohistochemistry
IL-7(2, etc)	interleukin 7 (2, etc)
IM	intramuscular
IME	important medical events
IMP	investigational medicinal product
IND	Investigational New Drug Application
INR	international normalized ratio
ION	Immune Oncology Network
IRB	Institutional Review Board
irAE	immune-related adverse event
iRECIST	immune-related Response Evaluation Criteria in Solid Tumors
IV	intravenous
IVIg	intravenous immunoglobulin
Kyn/Trp	Kynurenine and tryptophan
LCMV	lymphocytic choriomeningitis virus
LDH	lactate dehydrogenase
LFT	liver function test
mAb	monoclonal antibodies

MAD	maximum administered dose
MCC	Merkel cell carcinoma
MDS	myelodysplastic syndrome
MDSC	myeloid-derived suppressor cells
MED	maximum effective dose
MedDRA	Medical Dictionary for Regulatory Activities
MHC	major histocompatibility complex
MRI	magnetic resonance imaging
msIHC	multispectral IHC
MTD	maximum tolerated dose
NADA	neutralizing anti-drug antibodies
NCI	National Cancer Institute
NIT	NeoImmuneTech
NK	natural killer (cells)
NKT	natural killer T (cells)
NOAEL	no observed adverse effect level
NSCLC	non-small cell lung cancer
NT-I7	rh-IL-7-hyFc ka GX-I7
ORR	objective response rate
OS	overall survival
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PD	progressive disease
PD-1	programmed cell death protein 1
PD-L1	programmed death-ligand 1
PET	positron emission tomography
PFS	progression-free survival
Ph. EUR	European pharmacopeia
PI	Principal Investigator
PK	pharmacokinetic(s)
PR	partial response
PSA	prostate-specific antigen
PT	prothrombin time

PUVA	psoralen plus ultraviolet A radiation
Q3W	every 3 weeks
RCC	renal cell carcinoma
RECIST	Response Evaluation Criteria in Solid Tumors
rhIL-7	recombinant human IL-7
RP-HPLC	reverse-phase high-performance liquid chromatography
RP2D	recommended Phase 2 dose
SAE	serious adverse event
SAP	statistical analysis plan
SC	subcutaneous
SD	stable disease
SE-UHPLC	size-exclusion ultra-high-performance liquid chromatography
SFC	spot-forming cells
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic-pyruvic transaminase
SOC	system organ class
SOP	standard operating procedure
SST	serum-separating tube
TAA	tumor-associated antigen
TB	tuberculosis
TC	tumor cells
TCR	T-cell receptor
TEAE	treatment-emergent adverse event
TEMRA	terminally differentiated effector memory
TGF-β	transforming growth factor β
TIL	tumor-infiltrating lymphocyte
TME	tumor microenvironment
TREC	T cell receptor excision circles
TSH	thyroid-stimulating hormone
T-VEC	talimogene laherparepvec
Tx	treatment
UC	urothelial carcinoma
ULN	upper limit of normal

UV	ultraviolet
WES	whole exome sequencing