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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Wrangle JM, Velcheti V, Patel MR, et al. ALT-803, an IL-15 superagonist, in combination with nivolumab in patients with metastatic non-small cell lung cancer: a non-randomised, open-label, phase 1b trial. *Lancet Oncol* 2018; published online April 5. http://dx.doi.org/10.1016/S1470-2045(18)30148-7.

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The definitions of Dose Limiting Toxicity are described within this section and are categorized as Non-Immune-Related Adverse Events and Immune Related Adverse Events. The DLT period is the first 42 days for treatment with ALT-803.

Non-Immune-Related Adverse Events

Non-Immune-Related Adverse Events are DLTs and are defined as follows: during the first 28 days of the study treatment, any toxicity that is not clearly unrelated to study treatment administration that is of Grade 3 and does not resolve to Grade 1 or lower within a week despite the use of medical intervention, or that is of Grade 4, but with exceptions as follows:

- The following events occurring during the DLT period ARE also considered a DLT.
- Arrhythmia: Grade > 3 arrhythmia of any kind is a DLT.
- ALC ≥ 50,000/μL sustained for 14 days is a DLT.
- WBC ≥ 60,000/μL sustained for 14 days is a DLT.
- Hypotension of Grade 3 that persists for > 4 hours and requires hospitalization
- The following events occurring during the DLT period ARE NOT considered a DLT.
- Any Grade 3 or 4 lymphopenia, leukopenia and/or neutropenia that recovers within 14 days is not a DLT.
- Any Grade 3 or 4 thrombocytopenia with or without bleeding or anemia that recovers within 14 days is not a DLT.
- Nausea or vomiting controllable with anti-emetics within 72 hours is not a DLT.
- Hypotension (systolic pressure < 90 mm Hg) of Grade < 3 that is of limited duration (less than 72 hours) or can be managed with hydration measures as described in Section 6 is not a DLT.
- Hypotension that requires a precautionary admission for observation after grade 3 hypotension that persists for ≤ 4 hours is not a DLT.
- Grade 3 amylase and lipase elevations that recover within 14 days is not a DLT.

Immune-Related Adverse Events

The following Immune-Related Adverse Events are DLTs:

- Any Grade 4 irAE regardless of duration
- Any ≥ Grade 4 colitis regardless of duration
- Any Grade 3 or Grade 4 non-infectious pneumonitis regardless of duration
- Any Grade 3 irAE, excluding colitis and pneumonitis, that does not downgrade to ≤ Grade 2 within 3 days after onset of the event despite maximal supportive care including systemic corticosteroids or downgrade to ≤ Grade 1 or baseline within 14 days
- Any Grade 2 pneumonitis that does not resolve to ≤ Grade 1 within 3 days of the initiation of maximal supportive care
- Liver transaminase elevation higher than 8 × ULN or total bilirubin higher than 6 × ULN
- Any other toxicity that is greater than baseline grade, is clinically significant and/or unacceptable, and is judged to be a DLT by the Sponsor-Investigator
- The definition excludes the following conditions:
- Grade 3 endocrinopathy that is managed with or without systemic corticosteroid therapy and/or hormone replacement therapy and the subject is asymptomatic
- Grade 3 inflammatory reaction attributed to a local antitumor response (eg, inflammatory reaction at sites of metastatic disease, lymph nodes, etc) that resolves to ≤ Grade 1 within 4 weeks
- Concurrent vitiligo or alopecia of any AE grade

Site Name	Site Investigator	Patients Accrued
Medical University of South Carolina	John Wrangle	13
Cleveland Clinic	Vamsidhar Velcheti	6
University of Minnesota	Manish Patel	4

Baseline Characteristics of PD-1 mAb Immunotherapy Relapsed and Refractory	
Number of Subjects	11
Age, years, median (range)	62 (27-77)
Sex	
Male (%)	73
Female (%)	27
Race	
White	9 (82%)
Black	1 (9%)
Asian	1 (9%)
Smoking Status	
Never, No. (%)	5 (45%)
Former, No (%)	6 (55%)
Histology	
Squamous	2 (18%)
Non-Squamous	9 (82%)
ECOG, baseline	
0	6 (55%)
1	5 (45%)
PD-L1 Expression (%)	
0 - <1	5 (45%)
≥1 - <50	1 (9%)
>50	2 (18%)
Unknown	3 (27%)
Previous Treatments Regimens	1-9 (3)
1	1 (9%)
2-3	7 (64%)
>3	3 (27%)
Prior Platinum Based Chemotherapy (%)	11 (100%)
Prior Definitive Chemoradiation (%)	1 (9%)
Prior PD-1 mAb Immunotherapy, No. (%)	11 (100%)
Duration of prior PD-1 mAb Immunotherapy, range in months (median)	3-16 (5)
Mutations	
KRAS	2 (18%)
EGFR	1 (9%)
ALK Rearranged	0 (0%)
RET Rearranged	0 (0%)
ROS1 Rearranged	0 (0%)
BRAF	2 (18%)

Adverse events by dose cohort (n=21)

Adverse Events	6mcg/kg (N=3)			10 mcg/kg (N=3)			15mcg/kg (N=6)			20mcg/kg (N=9)		
	Grades			Grades			Grades			Grades		
	1-2	3	4-5	1-2	3	4-5	1-2	3	4-5	1-2	3	4-5
Constitutional												
Injection Site Reaction	2	0	0	3	0	0	5	0	0	9	0	0
Flu-like Symptoms	2	0	0	3	0	0	4	0	0	6	0	0
Fever	1	0	0	2	0	0	3	0	0	5	1	0
Fatigue	2	0	0	2	0	0	2	1	0	4	1	0
Anorexia	1	0	0	1	0	0	1	0	0	1	0	0
Chills	1	0	0	1	0	0	1	0	0	3	0	0
Dizziness	0	1	0	1	0	0	3	0	0	1	0	0
Gen. Muscle Weakness	0	0	0	0	0	0	1	0	0	1	0	0
Gastrointestinal												
Nausea	1	0	0	1	0	0	3	0	0	3	0	0
Constipation	0	0	0	1	0	0	3	0	0	0	0	0
Vomiting	0	0	0	1	0	0	0	0	0	2	0	0
Diarrhea	1	0	0	0	0	0	0	0	0	1	0	0
Dry Mouth	0	0	0	0	0	0	1	0	0	1	0	0
Mouth Sore	0	0	0	0	0	0	0	0	0	2	0	0
Respiratory												
Cough	1	0	0	1	0	0	0	0	0	2	0	0
Dyspnea	2	0	0	0	0	0	1	0	0	1	0	0
Cardiovascular												
Hypotension	1	0	0	2	0	0	0	0	0	1	0	0
Laboratory												
Lymphocytopenia	0	1	0	0	0	0	0	0	0	0	1	0
Increased Alk Phos	0	0	0	0	0	0	0	1	0	0	0	0
Anemia	0	0	0	0	0	0	0	0	0	0	1	0
Increased AST	0	0	0	0	0	0	0	1	0	0	0	0
Pain/Other												
Pain	0	0	0	1	0	0	3	0	0	3	0	0
Rash (NOS)	0	0	0	0	0	0	0	0	0	2	0	0
Headache	0	0	0	0	0	0	1	0	0	1	0	0
Back Pain	0	0	0	0	0	0	1	0	0	0	1	0
Depression	0	0	0	0	0	0	0	1	0	1	0	0
Abdominal Pain	0	0	0	0	1	0	0	0	0	0	0	0
Myocardial Infarction	0	0	0	0	1	0	0	0	0	0	0	0

Adverse events in patients with prior PD-1 blockade (n=11)

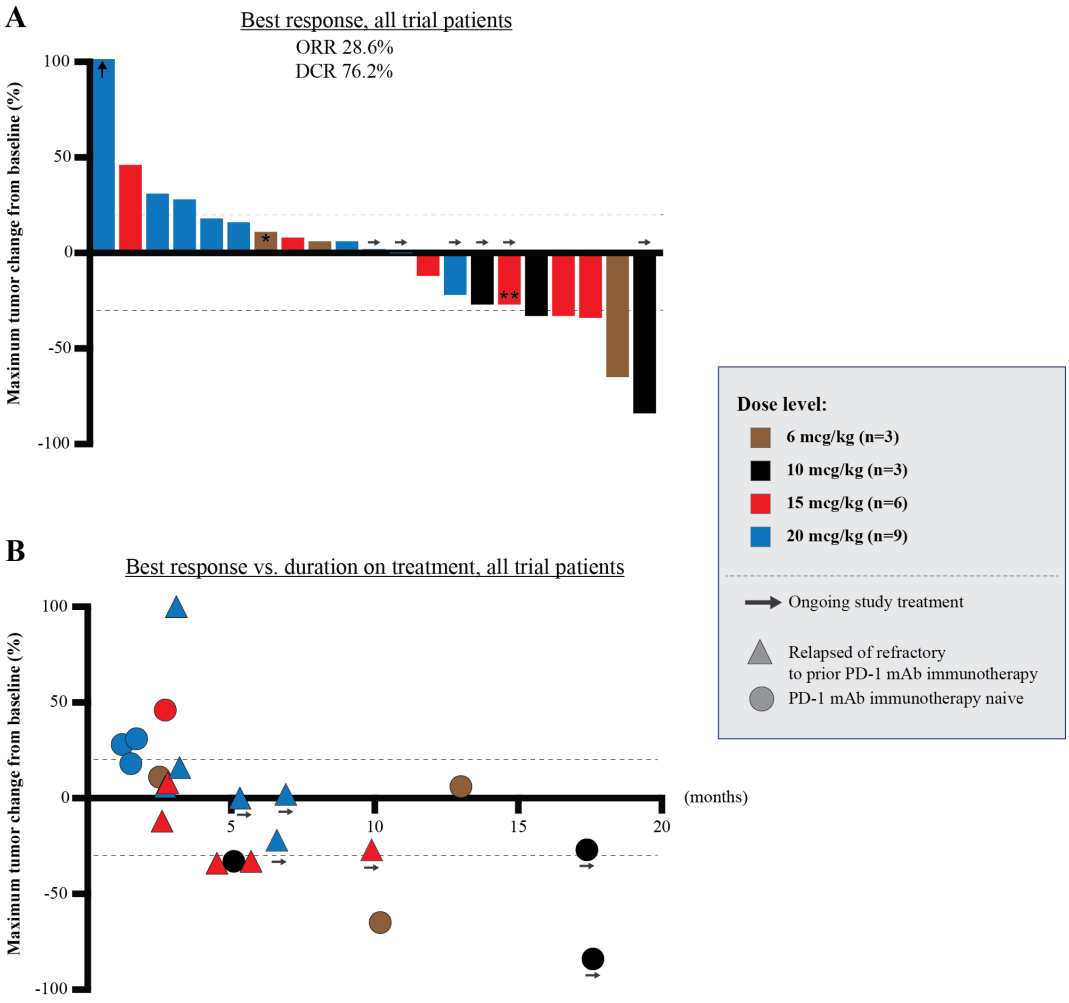
	Grades		
	1 or 2	3	4-5
Constitutional			
Injection Site Reaction	10 (90%)	0	0
Flu-like Symptoms	9 (81%)	0	0
Fever	4 (36%)	1 (9%)	0
Fatigue	5 (45%)	2 (18%)	0
Anorexia	2 (18%)	0	0
Chills	3 (27%)	0	0
Dizziness	3 (27%)	0	0
Gen. Muscle Weakness	1 (9%)	0	0
Gastrointestinal			
Nausea	5 (45%)	0	0
Constipation	2 (18%)	0	0
Vomiting	2 (18%)	0	0
Diarrhea	1 (9%)	0	0
Dry Mouth	1 (9%)	0	0
Mouth Sore	1 (9%)	0	0
Respiratory			
Cough	2 (18%)	0	0
Dyspnea	2 (18%)	0	0
Cardiovascular			
Hypotension	0	0	0
Laboratory			
Lymphocytopenia	0	0	0
Increased Alk Phos	0	1 (9%)	0
Anemia	0	1 (9%)	0
Increased AST	0	1 (9%)	0
Pain/Other			
Pain	4 (36%)	1 (9%)	0
Rash (NOS)	2 (18%)	0	0
Headache	1 (9%)	0	0
Back Pain	1 (9%)	0	0
Depression	1 (9%)	1 (9%)	0
Abdominal Pain	0	0	0
Myocardial Infarction	0	0	0

Best Response by Mutation Status	
Gene	Response Characteristics
KRAS (G12X)	6 patients total. 3 patients experienced stable disease, 2 partial response, and 1 progressive disease
BRAF (V600E)	2 patients total. 1 patient experienced a partial response and 1 progressive disease.
EGFR (Exon 20 ins.)	1 patient total. Ongoing stable disease for 18+ months, -27% tumor volume decrease.
EGFR (L858R)	1 patient total. Stable disease for 3 months.
RET rearranged	1 patient total. Progressive disease

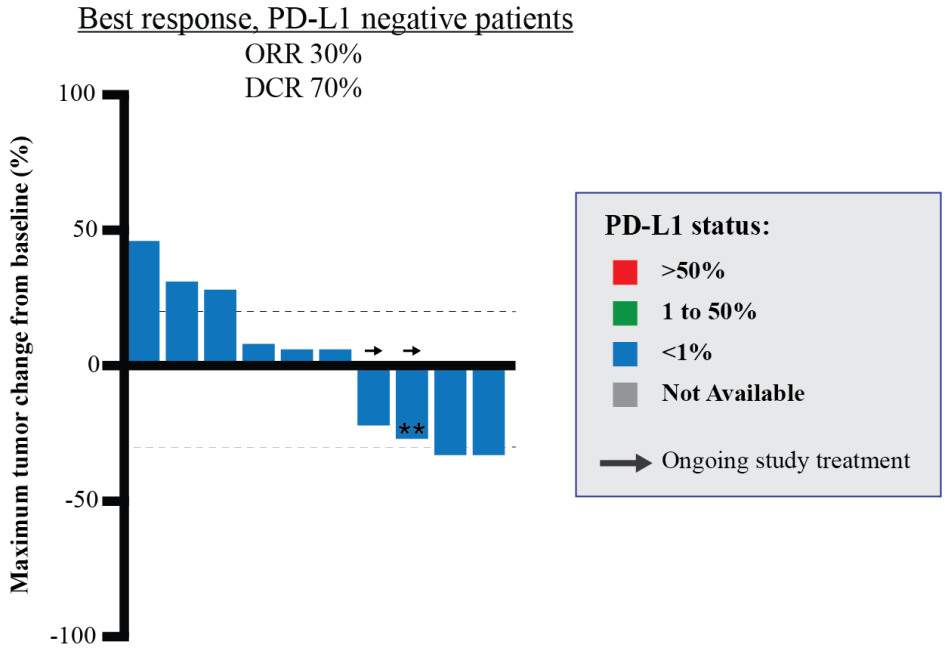
Anti-ATL-803 antibodies:

Patient #	C1W1D1	C1W2D1	C1W3D1	C1W5D1	C2W3D1	C3W2D1	C4W1D1
6mcg/kg							
1	NEG	NEG	NEG	NEG	NA	NA	NA
2	NEG	NEG	NEG	NEG	NEG	NEG	NEG
3	NEG	NEG	NEG	NEG	NEG	NEG	NEG
10mcg/kg							
4	NEG	NEG	NEG	POS (218)	NEG	NEG	NEG
5	NEG	NEG	NEG	POS (318)	POS (104)	POS (153)	POS (146)
6	NEG	NEG	NEG	NEG	NEG	NEG	NEG
15mcg/kg							
7	NEG	NEG	NEG	NEG	NEG	NEG	NEG
8	NEG	NEG	NEG	POS (134)	POS (110)	NA	NA
10	NEG	NEG	NEG	NEG	NEG	NA	NA
11	NEG	NEG	NEG	NEG	NA	NA	NA
12	NEG	NEG	NEG	POS (120)	NEG	NEG	NEG
13	NEG	NEG	NEG	NEG	NEG	NEG	POS (113)
20mcg/kg							
14	NEG	NEG	NEG	NEG	NA	NA	NA
15	NEG	NA	NEG	NEG	NA	NA	NA
16	NEG	NEG	NEG	NEG	NEG	NA	NA
17	NEG	NEG	NEG	NEG	POS (114)	POS (200)	POS (231)
18	NEG	NEG	NEG	NEG	NA	NA	NA
19	NEG	NEG	NEG	NEG	NEG	NA	NA
20	NEG	NEG	NEG	NEG	NEG	NEG	NEG
22	NEG	NEG	NEG	NEG	NEG	NEG	NA
23	NEG	NEG	NEG	POS (114)	NEG	NA	NA

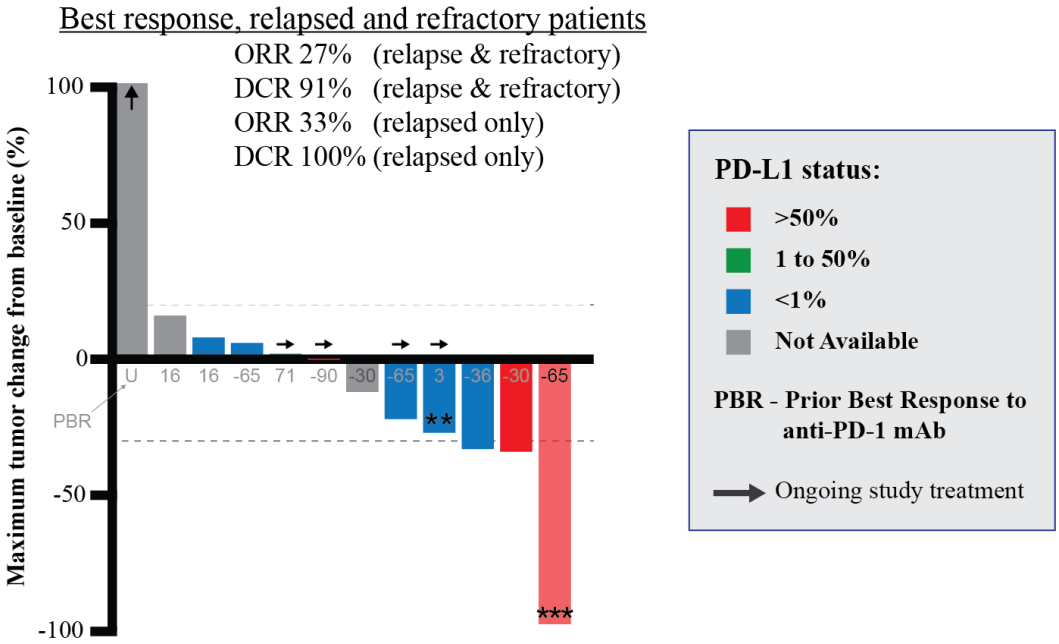
(NA, not available; for samples testing positive, the titer is in parenthesis)



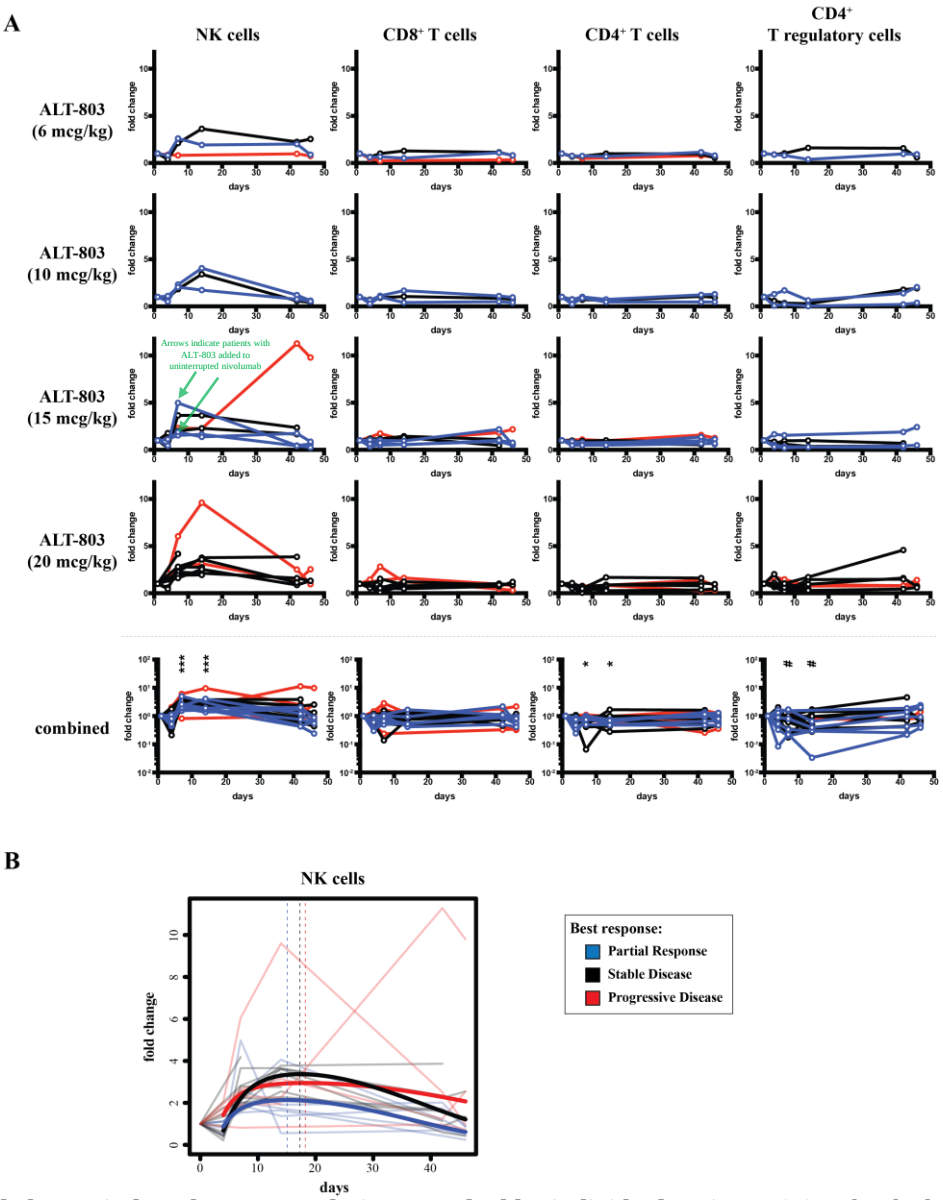
Clinical Efficacy of ALT-803 in combination with nivolumab displayed by dose cohort. Data graphed as shown in Figure 2 but color coded by dose cohort as indicated in the figure legend. (A) Best observed response for 21 patients treated with ALT-803 in combination with the FDA-approved dose of nivolumab. Arrows indicate ongoing therapy with study treatment. * Progression due to a new lesion. ** Target lesion decrease of -27%, but met RECIST 1.1 criteria for partial response. There is no significant association between best response (measured as a continuous variable) and dose ($p = 0.06$, Kruskal-Wallis test). (B) Best response by patient vs. duration of treatment on study. There is no significant association between time on study and dose ($p = 0.13$, Kruskal-Wallis test).



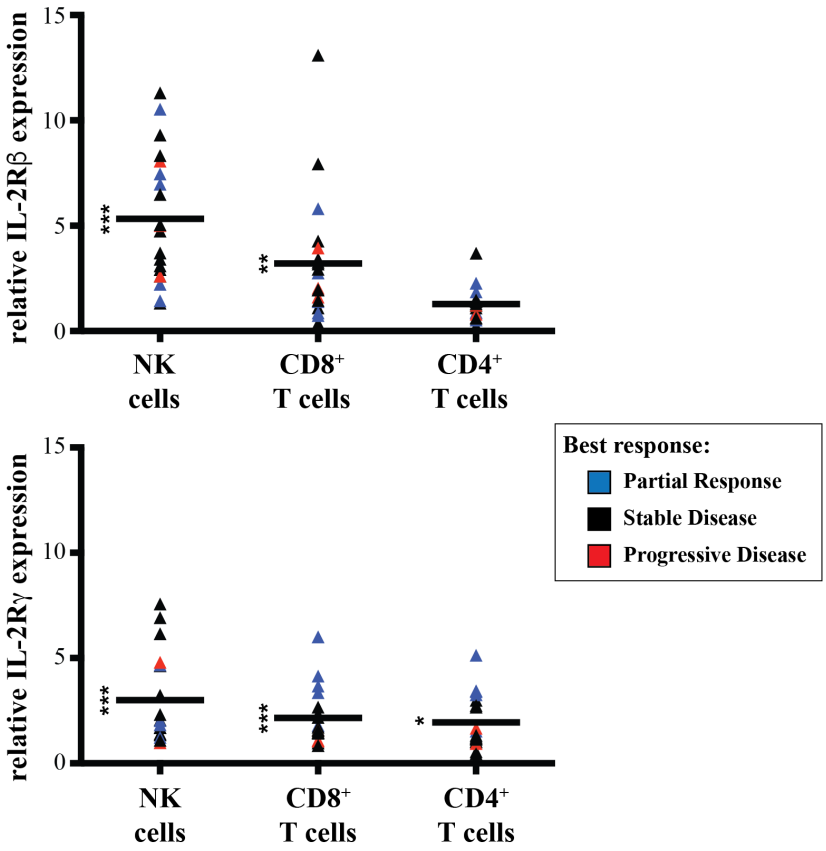
Clinical efficacy of ALT-803 in combination with nivolumab in patients with PD-L1 negative tumors. Best observed response for 10 patients with PD-L1 negative (<1%) tumors based on analysis of pre-treatment tumor biopsies. The overall response rate (ORR) was 30% and the disease control rate (DCR) was 70%. **Decrease of -27% qualified as RECIST 1.1 partial response by meeting the threshold for reduction in sum of largest diameters of target lesions.



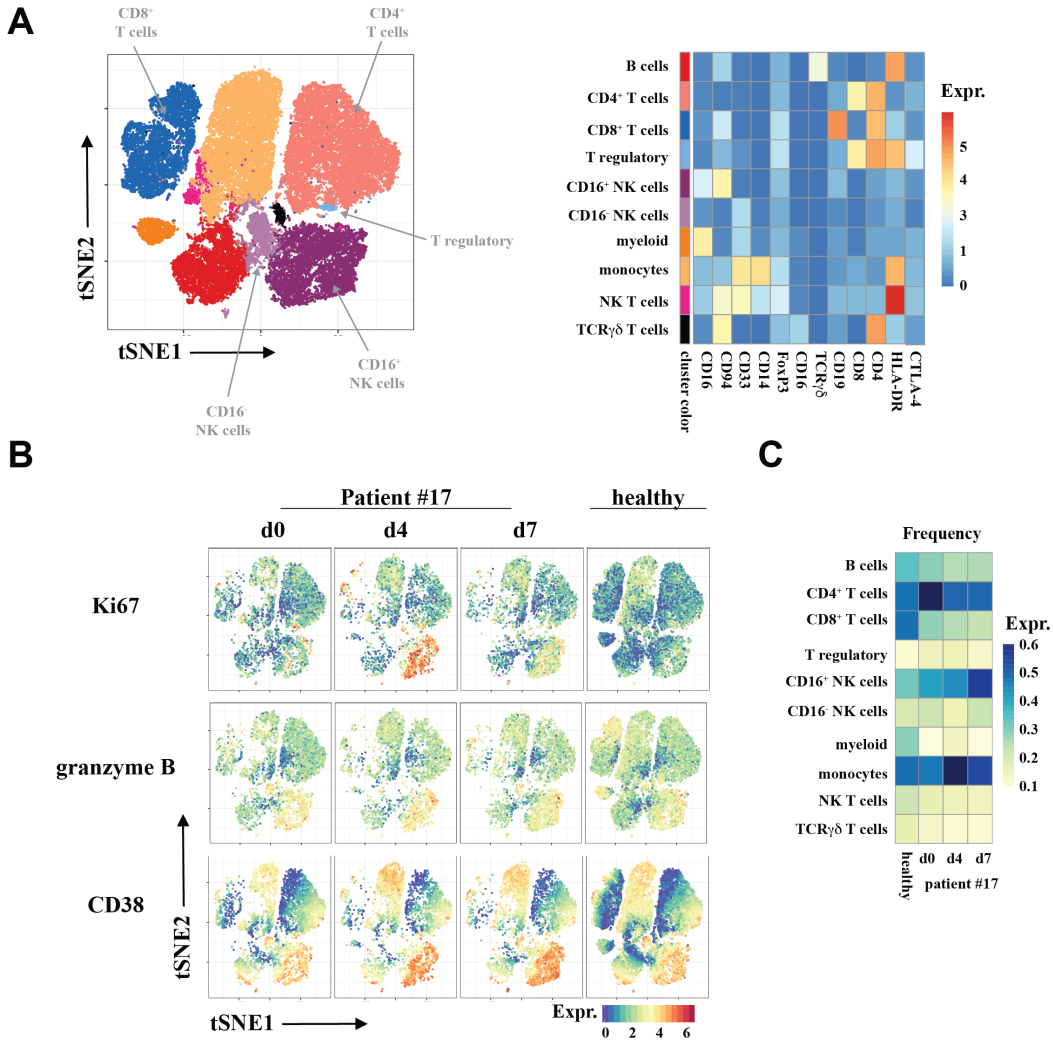
Clinical efficacy of ALT-803 in combination with nivolumab in patients after anti-PD-1 immunotherapy failure. Best observed response for 11 patients who were treated and progressed with single-agent nivolumab or pembrolizumab prior to study entry. The figure includes an additional patient (***, patient #3) who was treated in cohort 1 and responded (-65%), then progressed and went off trial, and then was retreated under a single-patient investigational new drug application with the dosing schema of cohort 3 and experienced another response (-100%) to trial therapy. Patient #3's second experience with nivolumab plus ALT-803 is not included in any efficacy analysis. The overall response rate (ORR) was 27% among anti-PD-1 immunotherapy resistant patients with a disease control rate (DCR) of 91%. PD-L1 status is indicated by color according to the legend. **Decrease of -27% qualified as RECIST 1.1 partial response by meeting the threshold for reduction in sum of largest diameters of target lesions.



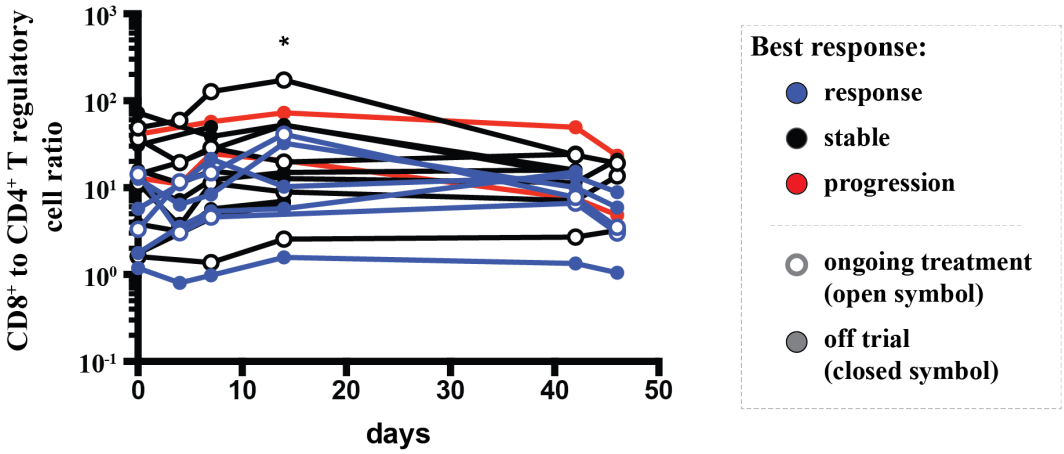
Fold change in lymphocyte populations graphed by individual patients. (A) Individual patient data by dose cohort as described for figure 3A. Also, included are $CD4^+FOXP3^+$ T regulatory cells graphed in a similar fashion for patients where this data is available. Day 7 and day 14 comparisons relative to baseline were performed to evaluate lymphocyte populations at time points proximal to cycle 1 therapy. NK cells increased significantly on days 7 (fold change (FC) = 2.4, $p < 0.0001$) and 14 (FC = 2.5, $p < 0.0001$) relative to baseline. Both $CD4^+$ T cells and $CD4^+FOXP3^+$ T regulatory cells decreased significantly on days 7 and 14 relative to baseline (FC = 0.62, $p = 0.0012$ and FC = 0.74, $p = 0.0039$, respectively, for $CD4^+$ T cells; FC = 0.63, $p = 0.012$ and FC = 0.54, $p = 0.014$, respectively, for $CD4^+FOXP3^+$ T regulatory cells). Changes in $CD8^+$ T cells at days 7 and 14 were not significant ($p = 0.20$ and $p = 0.58$, respectively). Fold changes that differ significantly from 1 are indicated in the 5th row which shows individual patients combined from all 4 dose cohorts graphed in log scale (# $p<0.05$, * $p<0.01$, *** $p<0.0001$, *t*-test). The two green arrows (15mcg/kg cohort) indicate patients in which ALT-803 was added to uninterrupted nivolumab. (B) Fold change estimates by response status for NK cells based on fitted mixed effects regression with time from pre-treatment modeled using a non-linear transformation using multivariable fractional polynomials. Individual patient data are shown using light-colored lines. Vertical dotted lines indicate the time of maximal predicted fold change based on the fitted model (responders, 15.1 days; stable disease, 17.3 days; progressive disease, 18.2 days).



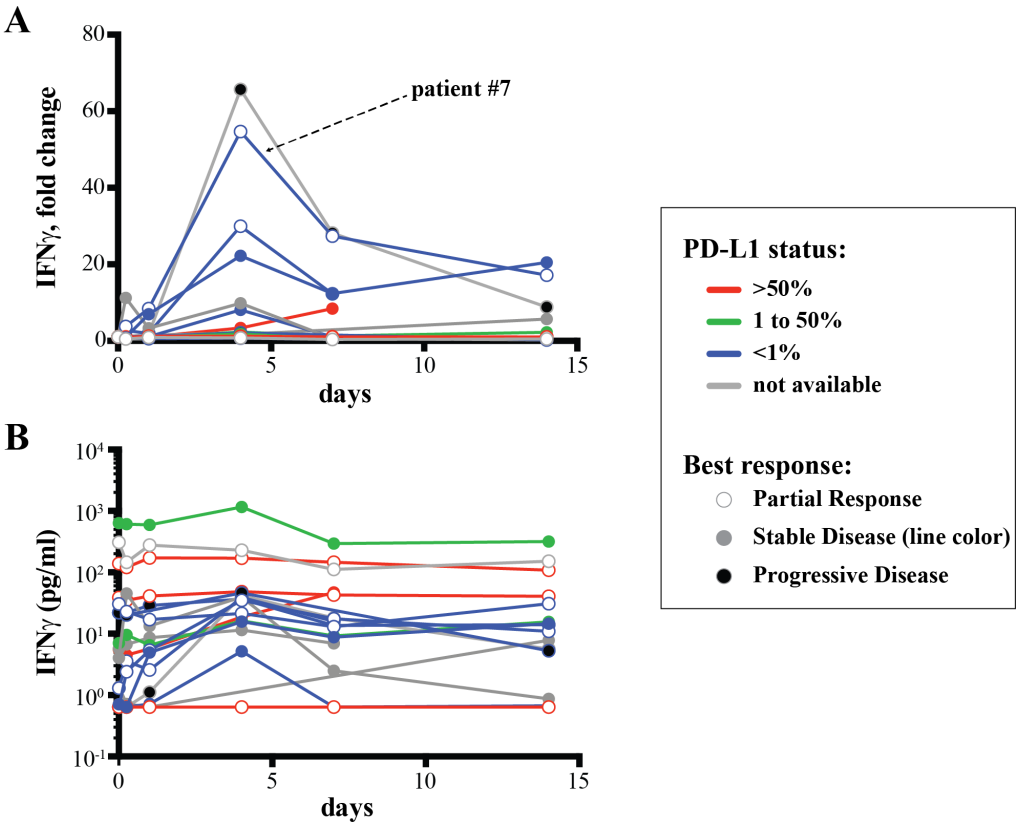
Relative expression of IL-2Rβ (CD122) and IL-2Rγ (CD132) on lymphocyte populations before therapy with nivolumab and ALT-803. Pre-treatment PBMC were analyzed by flow cytometry to identify NK cells ($CD56^+CD16^+CD3^-$), $CD8^+$ T cells ($CD8^+CD3^+$), and $CD4^+$ T cells ($CD4^+CD3^+$). Relative expression of IL-2Rβ (top) and IL-2Rγ (bottom) was determined by taking the MFI of each population divided by the MFI of cells negative for all markers. (For 4 patients, the negative control population had a small population of IL-2Rβ^{high} lymphocytes that was excluded from analysis.) Each symbol indicates one patient and colors indicate best response as depicted in the figure legend. Fold changes that differ significantly from 1 are indicated (* $p<0.01$, ** $p<0.001$, *** $p<0.0001$, *t*-test).



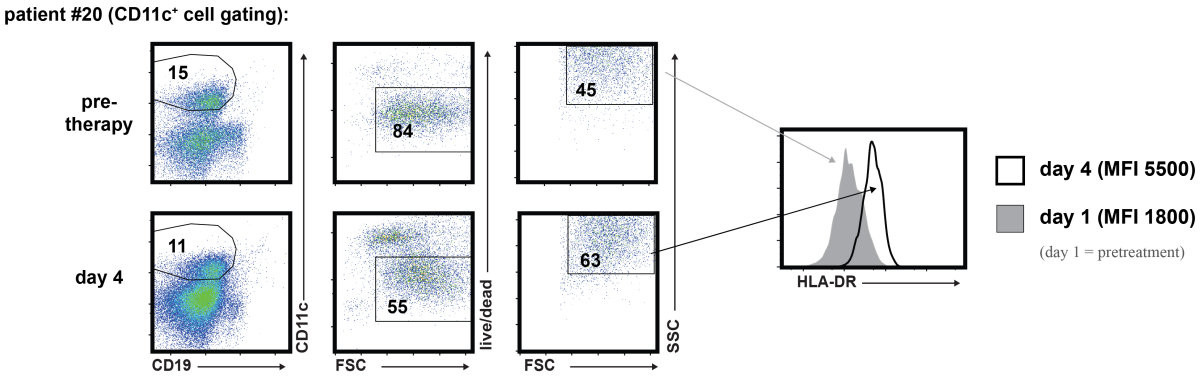
Mass cytometry analysis of cellular subpopulations in response to therapy. (A) Exemplified tSNE visualization of 50,000 cells (5,000 from each sample, which includes four healthy donors and the patient time points) colored based on main cell populations annotated by FlowSOM clustering and manual merging. The heatmap represents the median arcsinh-transformed marker expression. (B) Single cells from one exemplified patient before, and at days 4 and 7 after therapy colored by normalized expression of indicated markers (Ki67, granzyme B, and CD38) on the t-SNE map from 'A'. (C) The frequency of cell populations for a patient at baseline and at days 4 and 7 during therapy, shown using square-root frequency. Mean respective marker expression over four healthy donors served as a control.



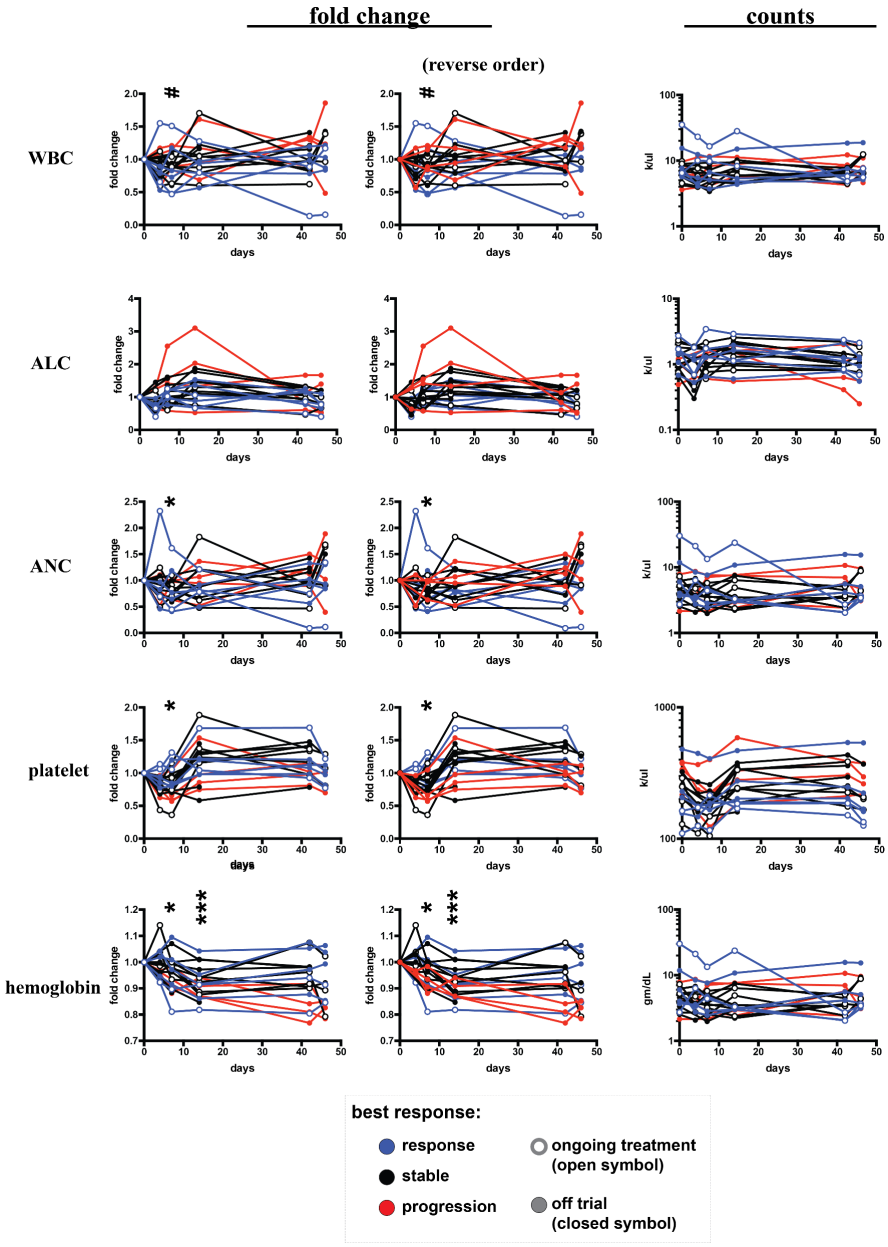
CD8 to T regulatory ratio graphed over time by dose response. Shown are ratio of $CD8^+$ T cells divided by $CD4^+FOXP3^+$ T cells graphed over time and color coded by best response. Day 7 and day 14 comparisons relative to baseline were performed to evaluate ratios at time points proximal to cycle 1 therapy. There was a significant increase in the day 14 ratio relative to baseline (FC = 1.80, p = 0.0038). Fold changes that differ significantly from 1 are indicated (*, $p<0.01$, paired *t*-test).



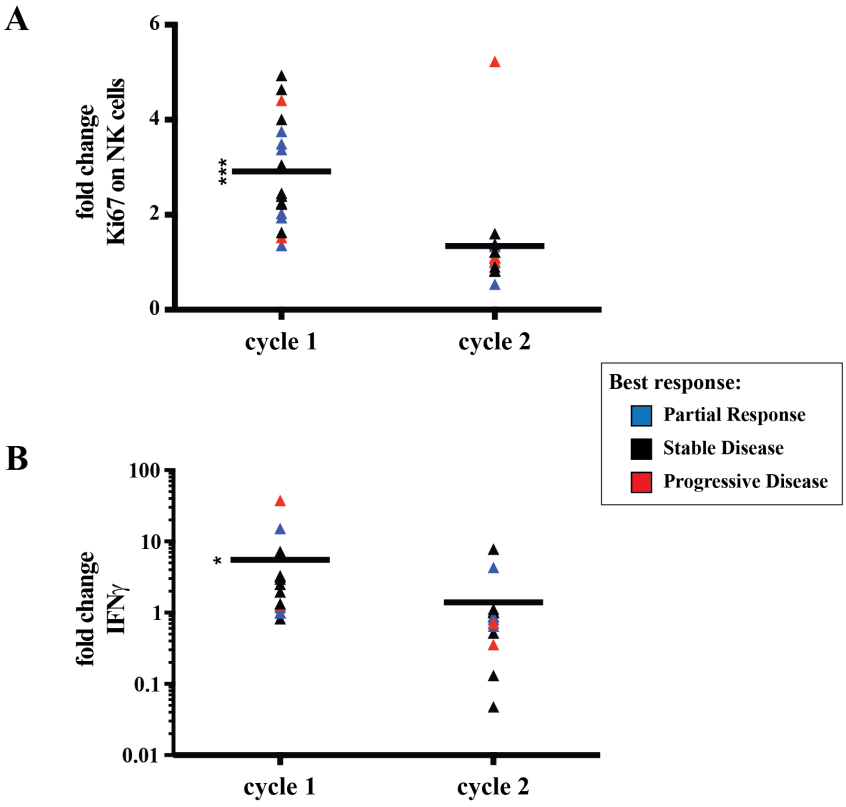
Induction of IFNγ in patients with PD-L1 negative tumors. A) IFNγ increase by fold change in serum over time graphed similarly to figure 3D and color coded by pre-treatment tumoral PD-L1 expression. B) As in 'A', except absolute serum values of IFNγ over time.



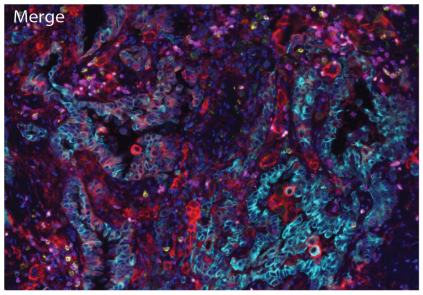
Gating strategy for determination of HLA-DR expression on CD11c$^+$ cells shown in figure 4E. PBMC from patient #20 (PD-L1 <1%) obtained prior to and four days after therapy was stained with a panel of markers including CD11c, CD19, and HLA-DR. Gating was performed as indicated (left to right). Fold change used in figure 4E was determining as the HLA-DR MFI in CD11c$^+$CD19$^-$ cells at day 4 divided by the pre-treatment (day 1) MFI value.



Complete blood counts (CBC) graphed over time. CBC values for WBC (white blood count), ALC (absolute lymphocyte count), ANC (absolute neutrophil count), platelets, and hemoglobin were graphed over time either by fold change (left) or raw counts (right). Best response is indicated by color and patients with ongoing treatment are designated with an open (white) symbol. Fold change was calculated based on the pre-treatment value. Day 7 and day 14 comparisons relative to baseline were performed to evaluate ratios at time points proximal to cycle 1 therapy. With the exception of ALC, all values were significantly smaller on day 7 relative to baseline ($p < 0.05$ for all CBC values except ALC). At day 14, only hemoglobulin was significantly reduced relative to baseline (FC = 0.92, $p < 0.0001$). Fold changes that differ significantly from 1 are indicated (#, $p<0.05$, *, $p<0.01$, and ***, $p<0.0001$, *t*-test).

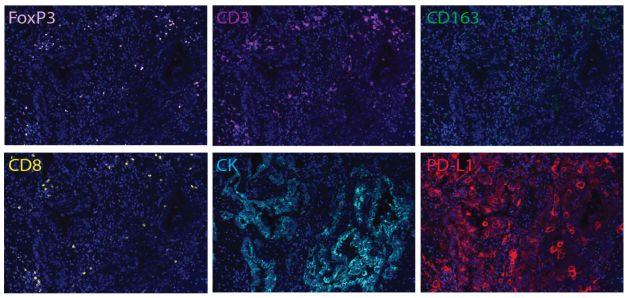


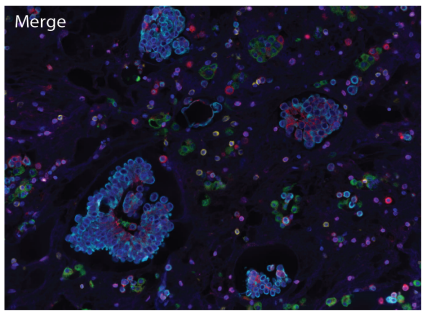
Therapy-induced changes in Ki67 and serum IFNγ levels are reduced during cycle 2 versus cycle 1. (A) Comparison of cycle 1 versus cycle 2 induction of Ki67 on NK cells. To determine fold change during cycle 1 and cycle 2, the MFI of Ki67 on NK cells at day 4 of each cycle was divided by the MFI of Ki67 on NK cells prior to therapy of each cycle. (B) Comparison of cycle 1 versus cycle 2 induction of serum IFNγ. To determine fold change during cycle 1 and cycle 2, the serum IFNγ levels at day 4 of each cycle was divided by the serum IFNγ levels prior to therapy of cycle 1. For panel A and B, each symbol indicates one patient and colors indicate best response as depicted in the figure legend. The bar indicates the mean. Fold change that differ from baseline are indicated (*, $p<0.01$ and ***, $p<0.0001$), *t*-test.



	PD-L1^{+}
tumor	66%
stroma (CD163^{+})	71%
stroma (CD163^{-})	49%

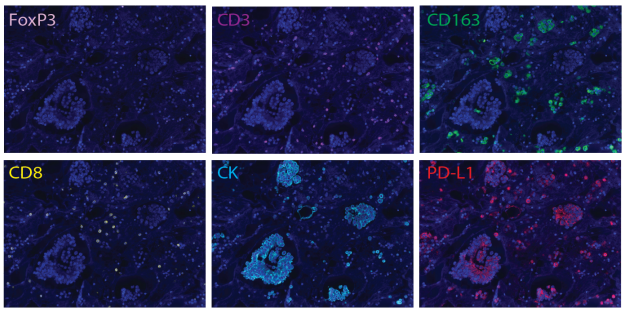
(26% of Stroma cells are CD163^{+})





	PD-L1^{+}
tumor	91%
stroma (CD163^{+})	27%
stroma (CD163^{-})	4%

(21% of Stroma cells are CD163^{+})



Multi-spectral imaging of pre-treatment biopsies from patients with NSCLC. Tumor biopsies (FFPE) from two patients were obtained prior to treatment. Representative single-color images (plus DAPI) of CD3, CD8, CD163, FoxP3, PD-L1, and cytokeratin (CK) staining and merged images are shown. Images were selected from representative "hot spots" of leukocyte infiltrates in each biopsy. Quantification was determined using InForm analysis software (PerkinElmer). All images are 200X.

Supplemental methods:

PBMC isolation and flow cytometry. Patient blood was drawn and processed within two hours by Ficoll gradient centrifugation to obtain peripheral blood mononuclear cells (PBMC). On days when therapy was administered, blood was obtained prior to drug administration. Fresh or cryopreserved PBMC samples were stained with a master mix of antibodies targeting for cell surface antigens including CD3 (UCHT1 or SK7 clone), CD4 (RPTA-4 clone), CD8 (SK1 clone), CD11c (B-ly6 clone), CD16 (3G8 clone), CD25 (BC96 clone), CD56 (HCD56 clone), CD57 (HNK-1 clone), CD122 (Mik-β3 clone), CD132 (TUGh4 clone), and HLA-DR (G46-6 clone), and intracellular antigens including EOMES (WD1928 clone), FOXP3 (236A/E7 clone), granzyme B (GB12 clone), Ki67 (20Raj1 clone), and T-bet (04-46 clone). For Ki67 and granzyme B, we used the BD Cytofix/Cytoperm Kit available from BD Bioscience, and for EOMES, FOXP3, and T-bet, we used the Foxp3/transcription factor staining buffer set (eBioscience). Cells were fixed with fixation buffer (Biolegend) and acquired on a BD LSRFortessa and analyzed using FlowJo software. For analysis of cryopreserved cells, the live/dead fixable blue dead cell stain (L23105, Molecular Probes) was used to exclude dead cells.

Serum cytokine analysis. The concentration of serum cytokines was assessed using bead based multiplex assays (Eve Technologies, Calgary, Canada). Samples were analyzed in duplicate and the fold change of the mean is shown.

Immunogenicity assays. Serum samples collected at the indicated time points for each subject were analyzed using qualified anti-ALT-803 and anti-nivolumab bridging ELISAs that use ALT-803 or nivolumab as the capture reagent and HRP-conjugated ALT-803 or HRP-conjugated nivolumab for detection. Samples are assayed in a dilution series. The samplewas classified as positive for anti-ALT-803 or anti-nivolumab antibodies if the average uncorrected OD of the post-dose sample was greater than 2X the average uncorrected OD of the corresponding pre-dose sample. The titer was calculated using the formula: (Uncorrected OD sample/ 2X (Uncorrected OD pre-dose)) X Dilution Factor.

ALT-803 pharmacokinetic analyses. Following blood collection, serum was isolated and immediately frozen, and later analyzed in batch for ALT-803. ALT-803 concentrations were determined in a qualified IL-15 ELISA assay (R&D Systems) using ALT-803 for generation of a standard curve.

TCR sequencing. Genomic DNA was isolated from PBMC using the QIAamp DNA Micro Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions at MUSC. DNA was then sent to Adaptive Biotechnologies for TCR immunosequencing. Immunosequencing of the CDR3 regions of human TCRβ chains was performed using the ImmunoSEQ^{TM} Assay. Extracted genomic DNA was amplified in a bias-controlled multiplex PCR, followed by high-throughput sequencing. Sequences were collapsed and filtered in order to identify and quantitate the absolute abundance of each unique TCRβ CDR3 region for further analysis as previously described [1-3].

Mass cytometry

After thawing, PBMCs were Live/Dead stained with 200μM Cisplatin-Pt-198 (Fluidigm) for 2 minutes at room temperature and fixed for 10 minutes with 2% PFA at 4°C. To achieve increased throughput and homogenous staining, metal cell barcoding was used[4,5]. Each sample was barcoded by using a three out of choice 6 barcode matrix[4]. The metal isotopes (Trace Sciences International, Richmond Hill, ON, Canada) used for barcoding were: 102Pd, 104Pd, 105Pd, 106Pd, 108Pd, and 110Pd. Metal barcoding reagents were prepared by combining 2 molar equivalents of isothiocyanobenzyl-EDTA (Dojindo Molecular Technologies, Rockville, MD) with 1 molar equivalent of metal chloride in ammonium acetate buffer (20 mM, pH 6.0). Chelated metal solutions were immediately lyophilized and dissolved in DMSO at 10 mM final concentration for long-term storage at -20°C. Barcoding reagents were titrated to achieve optimal labeling. A unique to each sample combination of exactly 3 metal cell barcoding reagents diluted in

300uL PBS-S (PBS+0.03% Saponin, Sigma-Aldrich), was added to each aliquot of cells in 200uL PBS-S isotopes and then incubated for 30 min at room temperature. Cells were washed twice with 1 mL PBS-S at 4°C. Barcoded cells were then combined in a single tube and washed with cell staining buffer (CSB, PBS + 0.5% BSA+2mM EDTA).

Surface proteins were stained with antibodies at 37°C for 20 minutes and for an additional 10 minutes at 4°C. Cells were washed in CSB and in order to perform intracellular staining, permeabilized using cytofix/cytoperm kit (BD) for 20 minutes on ice and stained with an intracellular antibody cocktail for 40 min on ice. Finally, cells were incubated over night with 250nM iridium intercalator (Fluidigm) to label cellular DNA. Subsequently, cells were washed with PBS followed by distilled water and resuspended in 10% EQ beads (Fluidigm) in distilled water. Mass cytometry acquisition was performed on a CyTOF2.1 (Helios) mass cytometer (Fluidigm). After acquisition, data was normalized by using the bead standard and the executable MATLAB normalizer application[6], dead cells and beads removed and debarcoded using Boolean gating using FlowJo software. All analyses on CyTOF data was performed after arcsinh (with cofactor equal to 5) transformation of marker expression. The bioinformatics workflow is based on FlowSOM[7] clustering and tSNE[8] visualization with manual annotation using heat maps of normalized marker expression and is published as a workflow article[9].

Multispectral imaging. Five micron sections from pre-treatment FFPE tumor biopsies were prepared and stained using PerkinElmer Opal Kit as previously described [10]. Immunohistochemistry was used to examine the presence and subtypes of leukocytes as well as PD-L1 expression in tumor samples and analyzed using multispectral imaging (Vectra; PerkinElmer). Slides were deparaffinized in xylene and alcohol. For fluorescence microscopy, slides were blocked with Ventana Antibody Diluent with Casein (Ventana) before staining with for the following antigens: CD3 (clone SP7; Spring Bioscience), PD-L1 (clone E1L3N; Cell Signaling), CD8 (clone SP16; Spring Bioscience; FoxP3 (clone 236A/E7; Abcam), CD163 (clone MRQ-26; Ventana), and pan-cytokeratin (clone AE1/AE3 clone, DAKO) followed by the anti-mouse or anti-rabbit secondary SuperPicTure Polymer Detection Kit (Life Technologies). The signals were amplified using the TSA OPAL kit (PerkinElmer). Microwave antigen retrieval in Citrate buffer (1X, pH 6.0) (Millipore) was done before each marker. Slides were stained with DAPI, mounted in Vectashield mounting medium (Vector Laboratories) and visualized using the Vectra Microscopy imaging system (PerkinElmer). Image analysis was performed using InForm analysis software (PerkinElmer) and representative regions were captured at 200X magnification.

Statistical considerations. Data analyses for immune markers were performed using R version 3.2.3 [11]. Primary endpoints for all variables were fold change (FC) relative to pre-treatment (baseline) values, unless otherwise indicated. All analyses were performed using (natural) logarithmic transformed FC values, resulting in improved conformity to normality assumptions based on graphical evaluations using histograms and quantile-quantile plots. Statistical evaluation of temporal trends in log-transformed FC values was conducted using linear mixed effect models with fixed effects for time from baseline (measured as a continuous variable) and dose cohort (6, 10, 15 or 20 mcg/kg). We also fit separate models with fixed effects for time and response status (response, stable disease or progression), time and PD-L1 status (low, medium or high), and time and PD-L1 status dichotomized as low versus not low (medium + high). All models included subject-specific random effects to account for the correlation among measures obtained from the same patient over time. We evaluated the linearity of time as a function of log-FC, and transformed time as appropriate using multivariable fractional polynomials [12]. We further explored interaction effects in each model to examine modification of the effect of time on log FC by dose cohort (response status or PD-L1 status). In separate analyses, we examined FC values at days 4 and 46 relative to baseline, and at day 46 relative to day 42. Specifically, we tested null hypotheses that log-FC values equal 0 (equivalent to tests of null hypotheses that FC = 1) based on one sample *t*-tests with two-sided $\alpha = 0.05$. We further performed pairwise comparisons of log-FC values across cell types using one-way ANOVA or Kruskal-Wallis test,

depending on conformity with normality. Comparisons between day 7 $CD8^+$ T cell-to-$CD4^+$ $FOXP3^+$ T regulatory cell ratio and baseline ratio was performed using a paired t-test. Associations between: best response (%) and dose cohort was evaluated using Kruskal-Wallis test; and response status (partial response, stable disease or progressive disease) and dose cohort was evaluated using Fisher's exact test. Statistical significance for all hypothesis tests was based on p-values < 0.05. We did not adjust for multiple comparisons.

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A PHASE IB/II STUDY OF NIVOLUMAB IN COMBINATION WITH ALT-803 IN PATIENTS WITH PRETREATED, ADVANCED OR METASTATIC NON-SMALL CELL LUNG CANCER

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PROTOCOL HISTORY:
Version 6.0 dated January 24, 2017
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Version 4.0 dated May 18, 2016
Version 3.0 dated January 15, 2016
Version 2.1 dated January 5, 2016
Version 2.0 dated November 12, 2015
Initial version 1.3 dated September 14, 2015

I have read the protocol entitled *A Phase Ib/II Study of Nivolumab in Combination with ALT-803 in Patients with Pretreated, Advanced or Metastatic Non-Small Cell Lung Cancer.*

I agree to conduct the study as detailed herein and in compliance with ICH Guidelines for Good Clinical Practice and applicable regulatory requirements and to inform all who assist me in the conduct of this study of their responsibilities and obligations.

_____
Principal investigator printed name

_____ _____
Principal investigator signature Date

_____
Participating Center Name

TABLE OF CONTENTS

DEFINITIONS OF TERMS USED

AE	adverse event
ASCT	allogeneic stem cell transplantation
ALC	absolute lymphocyte count
ALK	anaplastic lymphoma kinase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ANC	absolute neutrophil count
BMP	basic metabolic panel
BP	blood pressure
CBC	complete blood count
CCT	MUSC Core Facility (Center for Cellular Therapy)
CITN	Cancer Immunotherapy Trials Network
CHO	Chinese hamster ovary cells
CLS	capillary leak syndrome
CMP	complete metabolic panel
CNS	central nervous system
CR	complete response
CRF	case report form
CT	computer-assisted tomography
CTCAE	Common Toxicity Criteria adverse event
CTO	Clinical Trials Office
CTSA	Clinical and Translational Science Award
DLT	dose limiting toxicity
DSMC	Data Safety Monitoring Committee
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
eGFR	estimated glomerular filtration rate
EGFR	epidermal growth factor receptor
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HCC	Hollings Cancer Center
HGB	hemoglobin
HIV	human immunodeficiency virus
IB	Investigator Brochure
ICH	International Conference on Harmonisation
IIT	investigator initiated trial
IND	investigational new drug
INR	international normalized ratio
irAE	immune-related adverse event
IRB	Institutional Review Board
IV	intravenous
LTF	liver function test
MED	minimum efficacious dose
MTD	maximum tolerated dose
MRI	magnetic resonance imaging
MUSC	Medical University of South Carolina
NCI	National Cancer Institute
NHL	non-Hodgkin's lymphoma
NSCLC	non-small cell lung cancer
NYHA	New York Heart Association

OBD	optimum biological dose
ORR	objective response rate
OS	overall survival
PE	physical exam
PI	Principal Investigator
PFS	progression free survival
PK	pharmacokinetic
PLT	platelet count
PFT	pulmonary function test
PPI	proton pump inhibitor
PR	partial response
RD	recommended dose level
RECIST	Response Evaluation Criteria in Solid Tumors
REDCap	Research Electronic Data Capture
RCC	renal cell carcinoma
RP2D	Recommended Phase 2 Dose
SAE	serious adverse event
SD	stable disease
SIS-Unit	Sponsor Investigator Support Unit
Sub-Q	subcutaneous
ULN	upper limit of normal
WOCPB	woman of child bearing potential

1. PROTOCOL SYNOPSIS

Study Drug Name: ALT-803

Study Treatment Active agents: ALT-803, a "recombinant human super agonist interleukin-15 (IL-15) complex" (AKA, IL-15N72D:IL-15RαSu/IgG1 Fc complex)

Study Type: Interventional

Study Phase: Ib/II

Protocol Title: A phase Ib/II study of nivolumab in combination with ALT-803 in patients with pretreated, advanced or metastatic non-small cell lung cancer

Objectives: To define the safety and tolerability of escalating doses of ALT-803 used in combination with nivolumab and determine a recommended phase II dose of the combination in patients with pretreated, advanced or metastatic non-small cell lung cancer.

To define the response rate of ALT-803 added to nivolumab in patients with advanced and unresectable non-small cell lung cancer and to define the progression-free survival, overall survival, and duration of response of all treated patients.

To study the pharmacokinetics, immunogenicity, and immune correlates of ALT-803 in combination with nivolumab in treated patients.

Study Design: This is a Phase Ib/II, open-label, multi-center, competitive enrollment and dose-escalation study of ALT-803 in patients with pretreated, advanced or metastatic non-small cell lung cancer in conjunction with nivolumab.

The trial will consist of a phase Ib study with dose escalation of ALT-803 in combination with fixed dose nivolumab. The phase Ib portion of the study will generate the recommended phase II dose of ALT-803 to be used in combination with nivolumab. In the Phase Ib, five dose levels will be evaluated. The phase II portion of the study will consist of two arms, one for nivolumab naïve patients and another for patients progressing on nivolumab.

The study will be conducted in conformity with Good Clinical Practice (GCP).

Treatments: Nivolumab will be administered on Day 1 of every other week of each cycle beginning Week 1 Day 1 and continue until disease progression as defined in the protocol or unacceptable toxicity. ALT-803 will be administered over four 6-week cycles consisting of treatment on Day 1 of Weeks 1-5 followed by one week (W6) of rest.

The study treatment schema is as follows:

REGIMEN DESCRIPTION			
Agent	**Dose**	**Route**	**Schedule**
Nivolumab	240 mg	IV over 60 minutes[a]	Day 1 of Week 1, Week 3 and Week 5 of each cycle until progression
ALT-803	See Dose Escalation Schedule	sub-Q	1 Cycle = 6 weeks For Cycles 1-4 (ALT-803 + nivolumab) ALT-803 administered D1 of W1-5 W6 - Rest
a. +/-15 minutes			

Treated patients will have response evaluations every 6 weeks. Disease response and disease progression will be evaluated in this study using immune related (ir) RECIST criteria.

Enrolled patients will receive the study treatment at qualified cancer treatment centers with adequate diagnostic and treatment facilities to provide appropriate management of therapy and complications. The study drug ALT-803 will be administered subcutaneously. Injection sites should be rotated per institutional guidelines and each injection site separated by at least 1 inch. Nivolumab will be administered as outlined in the package insert.

Dose Escalation Phase:

A TiTE-CRM design will be used to guide dose escalation. The phase Ib portion of the trial will enroll up to 21 patients. The recommended phase II dose (RP2D) combination determined by the phase Ib portion of the study will be used to treat patients in the phase II portion of the study. There are five escalating dose levels of ALT-803.

Below are the planned dose levels of the study drug during the dose escalation phase of the study.

DOSE ESCALATION SCHEDULE		
Cohort	**ALT-803**	**Nivolumab**
-1	3 µg/kg	240 mg
1 (starting dose)	6 µg/kg	240 mg
2	10 µg/kg	240 mg
3	15 µg/kg	240 mg
4	20 µg/kg	240 mg

The Dose Limiting Toxicity definitions are categorized as Non-Immune-Related Adverse Events and Immune Related Adverse Events.

Non-Immune-Related Adverse Event DLTs are defined as follows: during the first 28 days of the study treatment, any toxicity that is not clearly unrelated to study treatment administration that is of Grade 3 and does not improve to Grade 1 or resolve within a week despite the use of medical intervention, or that is of Grade 4, with exceptions described in the study protocol. Immune-Related Adverse Events DLTs are defined in the protocol.

The DLT Observation Period is defined as the first 28 days of the study treatment.

Phase Ib Stopping Rules:

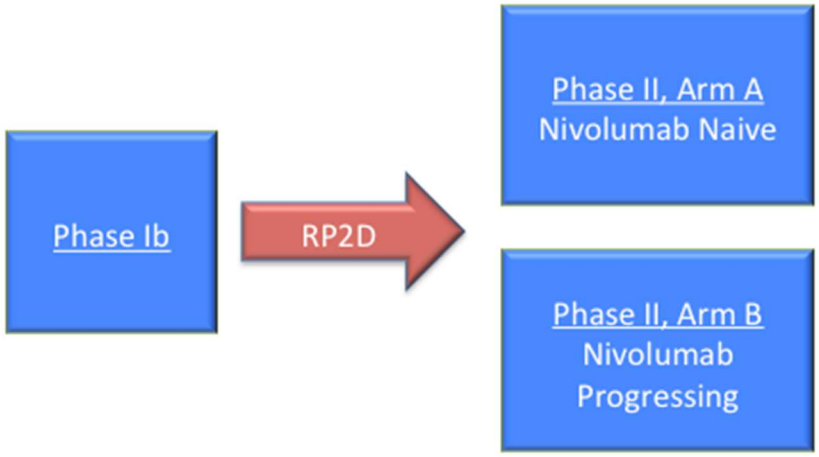
a) The sample size reaches 21

b) The recommended dose for the next cohort has already been given to 12 patients

c) The estimated DLT rate at dose -1 (3 μg/kg) is 0.40 or higher.

Expansion Phase

In phase II there will be two cohorts of patients: nivolumab naïve (cohort A) and nivolumab refractory/resistant (cohort B).



In each cohort, a predictive probability phase II design will be used with objective response rate (ORR) as the outcome (1). These designs will allow early stopping for futility beginning with the 9th patient and then after every 3rd patient treated on the trial. In this approach, the ORR is assumed to follow a binomial distribution, and we assume the prior on the ORR follows a beta distribution, leading to a beta-binomial posterior distribution for the number of responses. The predictive probability (PP) is the probability of concluding a positive result at the end of the trial based on the information that has accumulated from the patients treated thus far. If PP is high (e.g. PP > 0.90), it suggests there is strong evidence that the null hypothesis will be rejected at the trial's end. If PP is low (e.g. PP < 0.10), it suggests that it is unlikely that the trial will reject the null hypothesis by the end of the trial and the study should be stopped for futility. For the nivolumab naïve patients (cohort A), the null ORR is defined as 0.15 and an alternative ORR as 0.35. For the nivolumab refractory/resistant patients (cohort B), the null ORR is defined as 0.04 and an alternative ORR as 0.20. The stopping criteria for each arm of the phase II is specified in the protocol.

Evaluations: All patients will be evaluable for toxicity from the time of their first treatment with study drugs. Treated patients will have response evaluations every 6 weeks. Only those patients who have measurable disease present at baseline, have received at least one 6-week cycle of ALT-803 plus nivolumab therapy, and have had their disease re-evaluated will be considered evaluable for response. After end of study treatment, PFS, OS, and duration of response of all treated patients will be assessed at least every 3 months during years 1 and 2 and every 6 months during year 3. Prior to and during the treatment period, patient blood and serum samples will be collected to assess the pharmacokinetic profile and immunogenicity of the study drug, as well as for biomarker and cytokine analysis.

Population: Patients of 18 years of age and above with histologically or cytologically confirmed diagnosis of NSCLC who present with Stage IIIB/Stage IV disease (according to version 7 of the International Association for the Study of Lung Cancer Staging Manual in Thoracic Oncology), or recurrent disease following radiation therapy or surgical resection. Patients also need to have adequate cardiac, pulmonary, liver and kidney functions and have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.

Sample Size: The phase Ib portion of the trial will enroll up to 21 patients. A maximum of 40 patients (which includes any patients in the phase Ib trial who fit the eligibility criteria and are treated at the phase II dose level) will be included in the phase II portion of the study for the nivolumab, pembrolizumab or atezolizumab naïve (cohort A) group. A maximum of 30 patients (which includes any patients in the phase Ib trial who fit the eligibility criteria and are treated at the phase II dose level) will be included in the phase II portion of the study for the nivolumab, prembrolizumab or atezolizumab refractory/resistant (cohort B) group.

Primary Endpoints:

1) For the phase Ib portion of the study the primary objective is to define the safety and tolerability of escalating doses of ALT-803 used in combination with nivolumab and determine a recommended phase II dose of the combination.

2) For the phase II portion of the study the primary objective is to define the response rate of ALT-803 added to nivolumab in patients with advanced and unresectable non-small cell lung cancer.

Secondary Endpoints:

1) A secondary objective for both phases of the trial is to study the pharmacokinetics, immunogenicity, and immune correlates of ALT-803 in combination with nivolumab in patients with non-small cell lung cancer.

2) To define the progression-free survival, overall survival, and duration of response of all treated patients.

Pharmacokinetics & Biomarkers:	Fresh blood samples will be collected to assess immune cell number and phenotype. Serum samples will be collected for pharmacokinetics (PK) and immunogenicity of the study drug ALT-803. The same serum samples collected for PK analysis will be used to assess the serum levels of pro-inflammatory and immunosuppressive cytokines.
Monitoring Tests:	Blood samples for standard chemistry and CBC with differential will be obtained at screening and on each study treatment day. Blood samples for immunogenicity testing of anti-ALT-803 antibodies will be collected as outlined in section 13.
Response Assessment:	Treated patients will have response evaluations every 6 weeks. Disease response and disease progression will be evaluated in this study using irRECIST criteria.
Progression & Survival Assessment:	After end of study treatment, PFS, OS, and duration of response of all treated patients will be assessed at least every 3 months during years 1 and 2 and every 6 months during year 3.
Adverse Events:	All patients will be evaluable for toxicity from the time of their first treatment with study drugs. All patients will be monitored and evaluated for clinical toxicities during the treatment period and queried at each response assessment visit for AEs. The study center should report all SAEs and all events that trigger patient's study treatment discontinuation to the Sponsor-Investigator via phone, fax or email (or a combination) no more than 24 hours after learning of the event. The Sponsor-Investigator will use the information to manage and coordinate the dose escalation and patient enrollment. The Sponsor-Investigator will then inform Altor Bioscience and all of the participating clinical sites of the current dose level and the number of patients to be enrolled at that level, or of any patient enrollment suspension via phone, fax or email within 24 hours of learning of the event. The study center should report all other adverse events to the Sponsor-Investigator following the guidelines defined in the study protocol. All study treatment related AEs that are both serious and unexpected will be reported to the FDA in an expedited manner in accordance with 21 CFR §312.32

2. OBJECTIVES

2.1. PRIMARY OBJECTIVES

1) For the phase Ib portion of the study the primary objective is to define the safety and tolerability of escalating doses of ALT-803 used in combination with nivolumab and determine a recommended phase II dose of the combination.
2) For the phase II portion of the study the primary objective is to define the response rate of ALT-803 added to nivolumab in patients with advanced and unresectable non-small cell lung cancer.

2.2. SECONDARY OBJECTIVES

1) A secondary objective for both phases of the trial is to study the pharmacokinetics, immunogenicity, and immune correlates of ALT-803 in combination with nivolumab in patients with non-small cell lung cancer.
2) To define the progression-free survival, overall survival, and duration of response of all treated patients.

3. BACKGROUND AND RATIONALE

3.1. NON-SMALL CELL LUNG CANCER

NSCLC is the most frequently diagnosed human malignancy in the United States and the world. The chief risk factor for NSCLC is a history of cigarette use and incident cases of this disease have decreased with improved tobacco control efforts. Additionally, the incidence of non-smoking related NSCLC remains high. Development of traditional cytotoxic chemotherapy has been the focus of therapeutic trials in lung cancer until the discovery of the utility of tyrosine kinase inhibitors for cancers with mutations in the EGFR gene. Several years of intense interest in seeking new molecular abnormalities eligible for targeted therapy have yielded impressive results for the minority of patients with suitable mutations or translocations. On March 4, 2015, the FDA approved the anti-PD-1 immune checkpoint blocking antibody nivolumab for the treatment of NSCLC with squamous histology, ushering in the era of immunotherapy for the largest single cause of cancer morbidity and mortality in the world, lung cancer.

3.2. IMMUNOTHERAPY IN CANCER

The term immunotherapy encompasses a variety of therapeutic strategies unified by their ability to modify some aspect of the relationship between a tumor and the immune system. These therapies include bone marrow transplantation, cytokine therapy, adoptive cellular therapy, vaccination against tumor antigens, immune checkpoint blockade, and others (2-9). The ability of these therapies to mediate meaningful clinical responses in advanced malignancies has been recognized for years and engendered enthusiasm for their further development. Clinical responses, however, are far from universal and rarely complete (10-13). It is now apparent that tumor-induced immunosuppressive pathways are critical to tumor progression by inducing or maintaining lymphocyte dysfunction yielding ineffective anti-tumor immunity (14-16)(17). A treatment regimen capable of generating a robust anti-tumor immune response benefiting a higher percentage of patients with metastatic NSCLC than the ~20% who respond to nivolumab alone would be of immense use to the field of oncology.

3.3. IMMUNE CHECKPOINT INHIBITORS AS THERAPEUTIC AGENTS IN CANCER

Immune checkpoints are the receptor/ligand interactions by which cancers are able to induce dysfunction in tumor-reactive lymphocytes. In doing so, cancers are able to protect themselves from immune-mediated killing. Because this so-called “immune synapse” can be targeted by a monoclonal antibody which can block the repressive signal, lymphocyte dysfunction itself can be targeted. The immune-synapse encompasses a wide variety of receptor/ligand interactions, including the PD-1/PD-L1 pathway (18-24). The PD-1 gene was first isolated in 1992 and shown to a have a role in cell death (25). In 2002, Iwai et al. showed for the first time that the PD-1/PD-L1 pathway was important for T cell mediated tumor killing and tumor growth (26). These authors also showed that blockade of the PD-1/PD-L-1 pathway with an antibody against PD-L1 reduced tumor growth. This seminal work paved the way for many studies in which the PD1/PD-L1 pathway was demonstrated to have a critical role in tumor immunity.

The scope of the clinical significance of targeting T cell dysfunction is apparent when examining the breadth of solid tumors types for which responses have been observed in patients receiving antibodies targeting the PD-1/PD-L1 pathway. In addition to the classic

immunogenic tumors, melanoma and RCC, non-small-cell lung cancer, bladder cancer, Hodgkin's lymphoma, gastric cancer, ovarian and hepatocellular carcinoma all now are considered eligible to benefit from immunotherapy (8,18,27,28)(29-31). Nivolumab (anti-PD-1 mAb) has received FDA approval for patients with metastatic melanoma and squamous NSCLC and Pembrolizumab (anti-PD-1 mAb) is also approved for metastatic melanoma.

3.4. ANTI-PD1 IMMUNE-CHECKPOINT BLOCKADE IN NSCLC

The anti-PD1 antibody, nivolumab, was approved by the U.S. FDA on March 4, 2015 for use in squamous histology non-small cell lung cancer patients previously treated with a platinum-based doublet.

Topalian et al. reported experience of administrating nivolumab in a large phase I trial of 296 patients with several histologies (32). There were objective responses in 26 of 94 melanoma, 9 of 33 renal-cell carcinoma, and 14 of 76 non-small-cell lung cancer patients. Grade 3 or 4 drug-related adverse events occurred in 14% of patients. Interestingly, the authors found that in patients who had tumor biopsies positive for PD-L1 expression, there was a higher frequency of clinical responses. However, expression of PD-L1 in the tumor did not guarantee a response, and most patients still failed to respond to nivolumab. This study demonstrated that anti-PD-1 mAb could be administered in a safe manner and could induce durable clinical responses. In a parallel phase I study, Brahmer et al. reported the use of the anti-PD-L1 mAb (BMS-936559) in patients with multiple tumor types at advanced stages (28). The results of this trial were similar to the nivolumab trial with roughly 9% of patients experiencing grade 3 or 4 drug-related adverse events and objective response was observed in 9 of 52 melanoma, 2 of 17 renal-cell carcinoma, 5 of 49 non-small-cell lung cancer, and 1 of 17 ovarian cancer patients.

More recently, the results of Phase 2 and randomized Phase 3 studies of nivolumab and nivolumab versus docetaxel, respectively, in patients with advance, refractory squamous NSCLC have been published (33)(34). In the Phase 3 study, nivolumab was associated with a response rate of 20% (versus 9% for docetaxel), median progression free survival of 3.5 months (versus 6.0 months for docetaxel) and median overall survival of 9.2 months (versus 6.0 months for docetaxel). Treatment-related grade 3/4 adverse events were reported in 7% of the patients in the nivolumab group as compared to 55% of those in the docetaxel group. These results demonstrated that nivolumab treatment provided significantly better clinical benefit than docetaxel to patients with advanced, refractory squamous NSCLC, regardless of the tumor PD-L1 expression level.

3.5. CYTOKINES AS THERAPEUTIC AGENTS IN CANCER

Prior to the clinical development of immune checkpoint blockade as a clinical strategy, single-agent high-dose IL-2, a T-cell growth factor, demonstrated durable and complete responses (i.e., >10 years) in a small subset of patients with advanced renal-cell carcinoma and melanoma (35-38), suggesting that such patients might actually be "cured." These results led to FDA approval of IL-2 in advanced renal-cell carcinoma and melanoma in 1992 and 1998, respectively (39) . IL-2 drives T-cell proliferation and expansion and activates NK cells but also has properties that can dampen specific effector T cell–based immune responses, including the stimulation of activation induced cell death (AICD) and the proliferation and relative expansion of immunosuppressive regulatory T cells (Tregs). Additionally, a major challenge in the use of IL-2 has been tolerability. Used in sufficient doses, IL-2 administration requires hospitalization for intense cardiovascular, hemodynamic,

and renal monitoring. Moreover, although tested in a wide spectrum of preclinical models and human trials based on solid immunologic principles, low-dose, less toxic, IL-2– based regimens have shown limited potential for augmenting responses to other immunomodulatory agents, such as cancer vaccines and checkpoint blockers (40). As a result, there has been considerable interest in developing immunotherapeutic alternatives to IL-2 that provide stimulatory activity to patient's effector immune cells without causing severe systemic toxicities.

3.6. IL-15

Perhaps the most significant advance in developing an improved immunostimulatory molecule compared to IL-2 was the discovery of the related cytokine, IL-15 (41-43). While both IL-2 and IL-15 signal through the shared IL-2Rβγ receptor, which is responsible for intracellular signaling through the JAK/STAT, MAPK, and PI3K pathways in T cells and NK cells, only IL-2 engages the high affinity IL-2Rα. As IL-2Rα is expressed on Treg cells, IL-2 but not IL-15 induces the expansion of these immunosuppressive cells (44-46) . Because IL-15 can engage IL-2Rβγ without engaging IL-2Rα on Treg cells, it is thought to have an improved therapeutic index compared with IL-2 (47-50). Overall, IL-15 is a T-cell growth factor that (a) induces the activation and proliferation of $CD8^+$ T cells and NK cells, (b) maintains long-term memory T cells with relatively less effect on Tregs, and (c) inhibits activation-induced cell death (AICD). These advantages led IL-15 to be ranked first among various novel strategies by an NCI immunotherapy workshop as the agent with the greatest potential to impact clinical oncology (51). While the IL-15 agonist itself has tremendous potential, IL-15 bound to the soluble IL-15Rα yields an even more effective superagonist (52-56). Administration of IL-15/IL-15Rα-Fc cytokine complexes (such as ALT-803) have 50-200x increased biological activity compared with free IL-15, and remarkably improved anti-tumor activity when compared with free IL-15 (54-57).

Non-human primate studies and early phase human trials (58) have validated that administration of free recombinant human IL-15 (rhIL-15) cytokine can substantially increase the number of $CD8^+$ T cells and NK cells. The most effective regimen in non-human primates for increasing peripheral blood lymphocytes was continuous low-dose infusion of rhIL-15. A recent publication (59) reported that IL-15 at a dose of 20 μg/kg/day administered by continuous IV infusion for 10 days resulted in a massive (100- fold) expansion of peripheral blood lymphocytes with >50,000 per μL $CD8^+$ T cells in the peripheral blood. Daily bolus IV infusion was much less effective in non-human primates. In addition, daily bolus IV infusion of NCI rhIL-15 has a high peak and very short serum half-life. Daily bolus for 12 days increased T cells and NK cells in patients, but had substantial toxicity that appeared to parallel IL-2 toxicity (58). The presumption is that IL-15 is more likely to induce a generalized inflammatory syndrome (i.e., cytokine storm) when the serum levels exceed that which saturate the cell-bound IL-15Rα (Thomas Waldmann, unpublished observations). Thus, alternative routes of administration, currently under investigation at the NCI and CITN, might have a superior therapeutic index.

Subcutaneous infusion is a method that provides a lower peak level, but with an increase in serum half-life. Subcutaneous infusion in non-human primates with 20–40 μg/kg/day of rhIL-15 resulted in an approximate 10-fold expansion of $CD8^+$ T effector memory (TEM) cells. Subcutaneous administration of NCI rhIL-15 is being tested by the CITN as a simpler method to mimic the pharmacokinetics of continuous infusion.

In addition to the NCI rhIL-15 and ALT-803, Amgen produced glycosylated rhIL-15 from mammalian cells and tested it in non-human primates. Although no longer in development, Amgen rhIL-15 was shown to expand memory $CD8^+$ and $CD4^+$ T cells and NK cells in the peripheral blood, with minimal increases in $CD4^+CD25^+Foxp3^+$ Tregs. In rhesus macaques, daily IV administration of Amgen rhIL-15 (10–50 μg/kg) for 12 days expanded circulating $CD8^+$ TEM, leading to an inverted CD4/CD8 T-cell ratio by day 13. Daily subcutaneous administration of Amgen rhIL-15 resulted in persistently elevated plasma IL-15 levels with transient toxicity. In contrast, intermittent subcutaneous dosing of Amgen rhIL-15 every 3 days allowed for the clearance of IL-15 between doses, was well tolerated, and proved safe for more than 3 weeks (60).

3.7. ALT-803

3.7.1. Design and Structure

As indicated above, recent studies in mouse tumor models have demonstrated that the efficacy of IL-15 can be dramatically increased by pre-associating it with soluble IL-15Rα either in a single chain format or as an IL-15Rα-Ig Fc fusion (52,54,56,57,61-67). Thus, Altor constructed ALT-803. ALT-803 is a recombinant "human superagonist interleukin-15 complex" also referred to as "IL-15N72D:IL-15RαSu/IgG1 Fc complex." ALT-803 is expressed as a fusion protein complex in Chinese hamster ovary cells and purified by standard Protein A-based methods (56).

The structure of the conjugate is shown in the schema (Fig. A). ALT-803 is a soluble complex consisting of 2 protein subunits of a human IL-15 variant (64) associated with high affinity to a dimeric human IL-15 receptor α (IL-15Rα) sushi domain/human IgG1 Fc fusion protein (56). The IL-15 variant is a 114 amino acid polypeptide comprising the mature human IL-15 cytokine sequence with an Asn to Asp substitution at position 72 of helix C (N72D) (64). This substitution was found to increase the binding activity to the IL-2Rβ subunit and enhance the biological activity of IL-15. Thus, IL-15N72D represents a potent IL-15 superagonist.

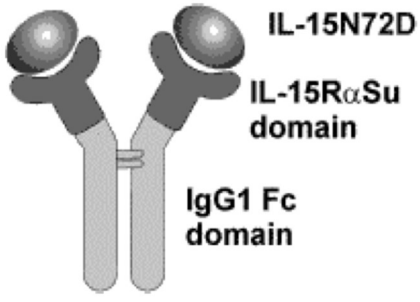


Figure A. Schematic drawing of the IL15N72D:IL-15RαSu/Fc complex consisting IL- 15N72D noncovalently associated with the dimeric IL-15RαSu/Fc fusion protein.

The human IL-15Rα sushi domain/human IgG1 Fc fusion protein of ALT-803 comprises the sushi domain of the IL-15Rα subunit (aa 1–65 of the mature human IL-15Rα protein) linked with the human IgG1 CH2-CH3 region containing the Fc domain (232 amino acids). Aside from the N72D substitution, all of the protein sequences in ALT-803 are human.

3.7.2. Pharmacokinetics and Pharmacodynamics of ALT-803 in Animals

ALT-803 has a dramatically longer serum half-life than unmodified rhIL-15 in CD-1 mice (Fig. B) (56). Comparative biodistribution studies with radiolabeled ALT-803 and IL-15 in mice also showed much greater retention of ALT-803 in the lymphoid organs (i.e., spleen, lymph nodes, thymus) than IL-15, which was rapidly cleared through the kidneys. The terminal elimination half-life of ALT-803 was approximately 7.6 hours in cynomolgus monkeys (68) and did not appear to significantly differ between dose levels. This is shorter than the 18- to 24-hour half-life observed in CD-1 mice (56).

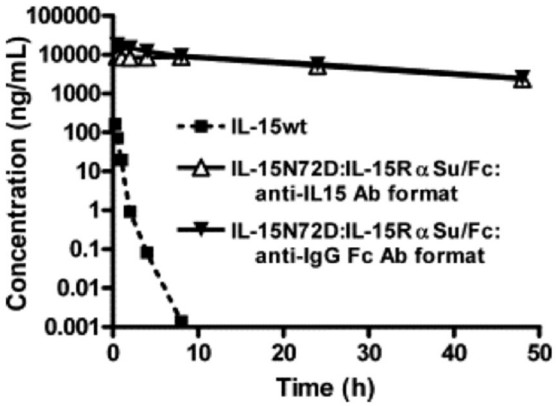


Figure B. Comparison of the pharmacokinetic profile of NH rhIL-15 and IL-15N72D:IL- 15RαSu/Fc complex after intravenous (IV administration in CD-1 mice. The anti-IL-15 Ab ELISA measures the concentration of IL-15wt (■). The anti-IL-15 Ab ELISA measures the concentration of the intact IL-15N72D:IL-15RαSu/Fc molecule (Δ), whereas the anti- human IgG Fc Ab ELISA measures serum concentration of the IL-15RαSu/Fc fusion protein (▼). The observed concentrations are represented by symbols and the model-fitted curves are represented by lines.

The biologic activity of ALT-803 is substantially higher than IL-15N72D or native IL-15 in proliferation assays using the IL-15 dependent 32Dβ cell line. Based on the dose response curves, the EC50s of ALT-803, IL-15N72D, and IL-15 wt were 5.6 pM, 38 pM, and 137 pM, respectively (Fig. C). Overall, ALT-803 exhibited a >10-fold lower EC50 than IL-15 alone for supporting IL-15–dependent cell growth, indicating that the complex provides highly potent biologic activity.

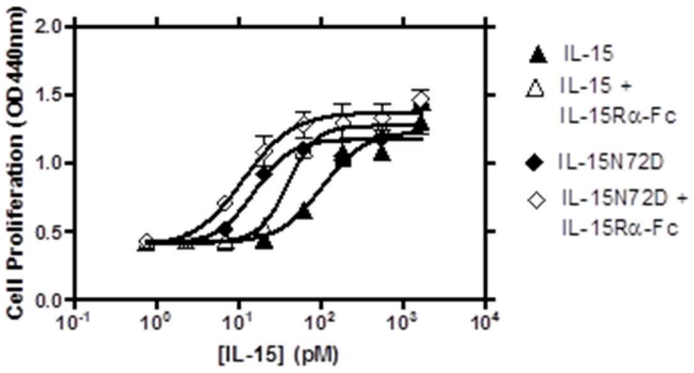


Figure C. Enhanced activity of ALT-803. The biological activity of the NIH rhIL-15 and IL- 15N72D proteins and ALT-803 (IL-15N72D + IL-15Rα-Fc) were compared in proliferation assays using the IL-15 dependent 32Dβ cell line. Based on the dose response curves, the EC50s of the IL-15 wt, IL-15N72D, and ALT-803 were 137 pM, 38 pM, and 5.6 pM, respectively.

The immunostimulatory effects of ALT-803 and IL-15 were further examined *in vivo* in mice. IL-15 was found to have no effect on the expansion of NK cells and $CD8^+$ T cells 4 days after a single i.v. dose of 0.28 mg/kg. In contrast, a molar equivalent dose (1 mg/kg) of ALT-803 significantly promoted NK cell and $CD8^+$ T cell proliferation in the blood (Fig. D) and spleen (data not shown), which led to lymphocytosis in blood and splenomegaly (56). A single-dose ALT-803 treatment at 0.2 mg/kg was found to stimulate $CD8^+$ T cells to secrete large amounts of interferon-γ and promote rapid expansion of $CD8^+CD44^{high}$ memory T cells in C57BL/6 mice. These memory $CD8^+$ T

cells exhibited ALT-803–mediated upregulation of NKG2D but not PD-1 or CD25 on their surfaces. ALT-803–activated $CD8^+$ memory T cells also exhibited nonspecific *in vitro* cytotoxicity against tumor cells (67). Dose response studies showed that a single treatment of ALT-803 at as low as 0.032 mg/kg was capable of stimulating immune responses of C57BL/6 mice. Multi-dose treatment with ALT-803 (1.0 and 0.2 mg/kg weekly for 4 weeks) also resulted in dose dependent increases in blood white blood cell (WBC) counts and spleen and lymph node weights. These immunostimulatory effects diminished but were still present 2 to 4 weeks after dosing (68).

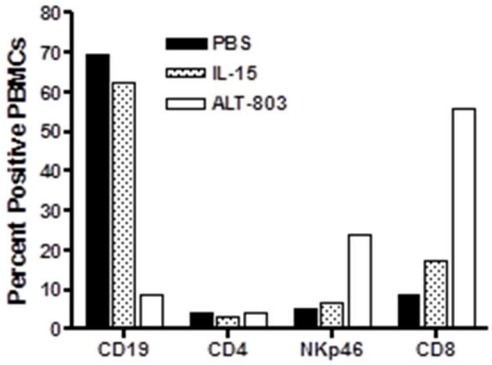


Figure D. ALT-803 stimulated $CD8^+$ T cells and NK cells responses *in vivo*. C57BL/6 mice (5 mice per group) were injected IV with a single dose of ALT-803 at 1 mg/kg, IL-15 at 0.28 mg/kg (molar equivalent dose), or PBS as a negative control. The percentage of B cells (CD19), CD4 T cells (CD4), NK cells (NKp46) and CD8 T cells (CD8) were determined in PBMCs 4 days after injection.

The pharmacodynamic profile of ALT-803 has also been assessed in a multidose toxicology study in cynomolgus monkeys (68). ALT-803 induced an increase in WBC counts after a single dose and 4 weekly doses of 0.1 mg/kg. Blood lymphocytes were elevated in study animals after a single dose and 4 weekly doses of 0.03 and 0.1 mg/kg ALT-803. Dose-dependent increases in $CD4^+$ and $CD8^+$ T-cell and NK-cell counts were observed (Fig. E), although the degree of these changes appeared to be less than those observed in mice treated with comparable doses. Blood leukocyte and lymphocyte counts of the ALT-803-treated monkeys returned to control levels after a 2-week.

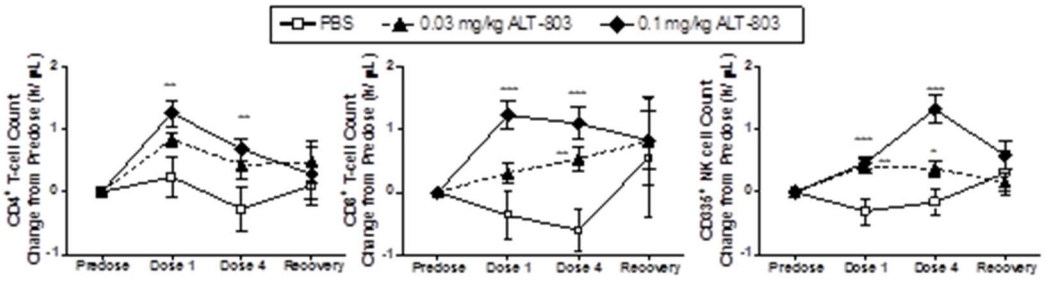


Figure E. Effects of ALT-803 on lymphocyte subsets in cynomolgus monkeys: Cynomolgus monkeys were treated with 0.03 and 0.01 mg/kg ALT-803 or PBS with 4 weekly IV doses with a 2 week recovery period. Lymphocyte subsets in the blood were assessed prior to the first dose, 4 days after the first and fourth doses, and after the 14 day recovery period.

3.7.3. Non-clinical Toxicology of ALT-803

In addition to the pharmacodynamic profile, the toxicity of ALT-803 was assessed in healthy C57BL/6 mice injected i.v. with 0.1, 1.0, or 4.0 mg/kg ALT-803, or PBS weekly for 4 consecutive weeks. Mice receiving 4.0 mg/kg ALT-803 exhibited signs of toxicity

(*i.e.*, weight loss, hair loss) and mortality between 4 - 20 days following treatment initiation. Post-mortem necropsy did not determine the primary cause of death but observations (*i.e.,* pulmonary edema, and enlarged lymph nodes and spleen) were consistent with cytokine-induced inflammatory responses (38,39). Treatment-related mortality was not observed in mice treated with 1.0 or 0.1 mg/kg ALT-803 (n = 50/dose level). As indicated, dose-dependent increases in spleen weights WBC counts were seen 4 days after the last dose of ALT-803. Of the WBCs, absolute counts for lymphocytes, neutrophils, and monocytes all increased over 8-fold in 1.0 mg/kg ALT-803-treated mice 2 weeks and 4 weeks after treatment compared to controls. Neutrophil counts remained elevated in 1.0 mg/kg ALT-803-treated mice but lymphocyte counts returned to control levels. Histopathological analysis verified ALT-803 dose-dependent stimulation of immune cell infiltration and hyperplasia in the spleen, liver, thymus, kidney, lungs and lymph nodes 4 days after the last dose, and to a lesser degree 2 and 4 weeks after treatment. The results of these studies define the tolerable dose of multidose ALT-803 treatment of up to 1 mg/kg in mice in a weekly dosing regimen for 4 weeks.

Cynomolgus monkeys receiving 4 consecutive weekly injections of ALT-803 at 0.1 and 0.03 mg/kg or PBS (control) did not exhibit any significant differences in mean body weights or any other dose-related clinical or behavioral observations among the groups. Additionally, organ weights were not significantly different in ALT-803-treated animals compared to controls. As indicated, the most biologically relevant changes observed following weekly ALT-803 treatment were dose-dependent increases in peripheral blood WBC and lymphocyte counts. An increased lymphocytic infiltration and proliferation in the liver, kidney and lungs was noted 4 days after the last dose of ALT-803. Clinical chemistry at this time point showed a decrease in serum albumin in the high-dose ALT-803 group compared to controls, which may be a consequence of inflammatory responses in the liver. However, serum liver enzyme levels were not elevated in ALT-803-treated animals compared to controls. Bone marrow hyperplasia was observed in most animals and increased in severity in the high-dose ALT-803-treated group. Histopathological changes of affected organs in the ALT-803-treated groups were reduced in incidence and severity by 2 weeks post-treatment and were consistent with findings in the control animals. Generally, the ALT-803-mediated effects on peripheral blood and tissue lymphocytes observed in this study are consistent with transient responses reported for non-human primates treated with IL-15 twice weekly at up to 0.1 mg/kg or daily at 10 to 50 μg/kg (15,26,27).
Eight of 20 animals in the ALT-803 treatment groups developed detectable anti-ALT-803 antibodies after 3 or 4 doses of the study drug. The pharmacologic consequences of these responses are unclear since there were no post-dosing allergic reactions or effects on ALT-803-mediated responses in animals that developed elevated levels of anti-ALT-803 antibodies.

3.7.4. Anti-tumor Activity of ALT-803 Monotherapy in Mouse Models

Initial studies compared the antitumor activity of ALT-803 and IL-15 against sub-Q B16F10 melanoma tumors or CT26 colon carcinoma metastases, which have previously been shown to be sensitive to IL-15-based therapies (22,36). Treatment of B16F10 tumor-bearing mice with 0.056 mg/kg IL-15 on days 1 and 8 failed to affect tumor growth. In contrast, equivalent dosing with IV ALT-803 (0.2 mg/kg) significantly inhibited growth of B16F10 tumors compared to the IL-15 or PBS (control) treatment

groups (Fig. F). These results are comparable to previous studies that showed superior efficacy of pre-associated IL-15:IL-15Rα complexes against s.c. and metastatic B16 tumors (16,22).

Similarly, ALT-803 treatment resulted in significant improvement of antitumor activity in BALB/c mice bearing CT26 tumor metastases compared to IL-15 treatment or PBS (Fig. F.B). In this study, IL-15 was administered at 0.25 mg/kg for a total of 10 doses over a 2-week period, resulting in modest improvement in survival of CT26 tumor-bearing mice compared to the control group. Treatment with 4 doses of 0.2 mg/kg ALT-803 provided significantly better survival benefit than either IL-15 or PBS in this model. Notably, this enhanced efficacy was observed with an 11-fold lower cumulative dose of ALT-803 compared to IL-15, based on cytokine molar equivalence.

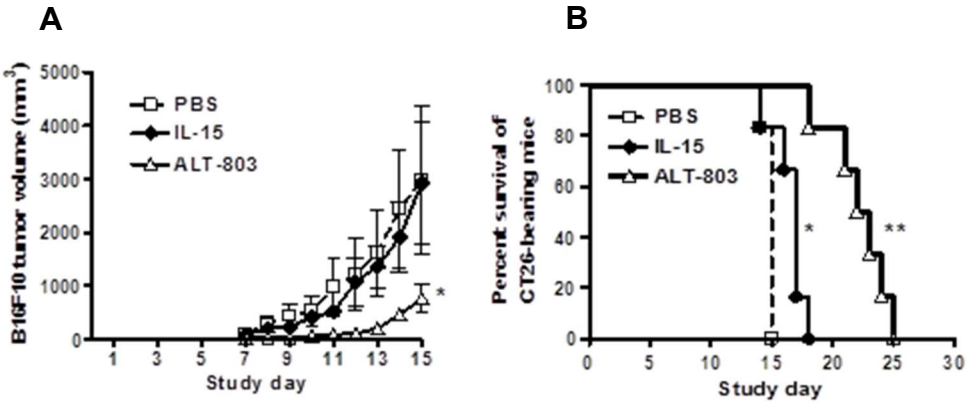


Figure F. Comparative antitumor activity of ALT-803 and IL-15 in immunocompetent mice bearing solid tumors. (*A*) Changes in tumor volume of subcutaneous B16F10 melanoma tumors in C57BL/6 mice treated with IV PBS (open squares), 0.2 mg/kg ALT-803 (open triangles), or 0.056 mg/kg IL-15 (IL-15 molar equivalent dose of 0.2 mg/kg ALT-803) (closed diamonds) on days 1 and 8 post-tumor injection. Data points are expressed as mean + SE (n = 5 mice/group). (*B*) Survival curves of BALB/c mice (n = 6/group) injected IV with CT26 colon carcinoma cells and subsequently treated with IV 0.25 mg/kg IL-15 (closed diamonds) on study days 1 to 5, and 8 to 12, or with 0.2 mg/kg ALT-803 (open triangles), or PBS (open squares) on study days 1, 4, 8, and 11.

To conduct efficacy studies in hematologic tumor models, highly tumorigenic myeloma lines 5T33P and MOPC-315P were derived from the well-characterized 5T33 and MOPC-315 parental lines, respectively (6). Following IV inoculation of syngeneic mice, these cells were found to populate the BM and cause paralysis. Tumor development in 5T33P-bearing C57BL/6 mice and MOPC-315P-bearing BALB/c mice was assessed by staining myeloma cells in isolated BM cell preparations for intracellular 5T33P-specific IgG2b and MOPC-315P-specific IgA paraproteins. In C57BL/6 mice, IgG2b paraprotein-positive myeloma cell levels increased to over 20% of the total BM cells by 21 days after 5T33P tumor cell inoculation.

A single IV treatment of ALT-803 (0.2 mg/kg) had a marked effect on 5T33P cells in the BM of mice with well-established tumors, providing >90% reduction in BM $IgG2b^+$ myeloma cells 4 days after treatment compared to controls. However, a molar equivalent

dose of IL-15 was much less effective and only reduced the percentage of BM 5T33P cells by 53% compared to PBS-treated mice. Dose response studies indicated that a single dose of ALT-803 at as low as 0.05 mg/kg was capable of reducing 90% of the BM 5T33P myeloma cells. 5T33P-bearing C57BL/6 mice treated with a single 0.2 mg/kg dose of ALT-803 also showed significantly increased survival when compared to PBS-treated mice (Fig. G).

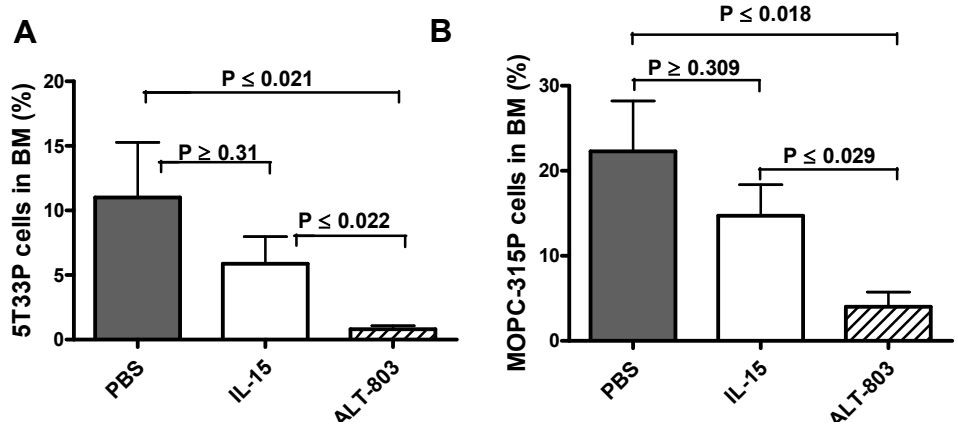


Figure G. Effect of ALT-803 or IL-15 on myeloma cells in BM of 5T33P or MOPC-315P bearing mice. Female mice (5 mice/group) were injected IV with 5T33P (*A*) or MOPC-315P (*B*) myeloma cells (1×10^7/mouse) on day 0. ALT-803 (0.2 mg/kg), IL-15 (0.056 mg/kg, an IL-15 molar equivalent dose to 0.2 mg/kg ALT-803), or PBS (dose volume equivalent) was then administered as a single IV injection on day 15 (5T33P) or 14 (MOPC-315P). Bone marrow cells were collected 4 days after study drug treatment. The cells were then stained with PE-conjugated rat anti-mouse IgG2b or IgA Ab to evaluate the percentage of 5T33P or MOPC-315P cells in BM, respectively. The plotted values represent the mean ± SE; *P* values are presented.

Additionally, 2 weekly injections of ALT-803 provided a significant survival benefit in this model, such that 80% of the ALT-803-treated mice survived over 190 days compared to 100% paralysis of the control group with a median survival of 29 days. Similar studies in BALB/c mice bearing well-established MOPC-315P tumors confirmed that treatment with ALT-803, but not IL-15, resulted in a significant decrease in BM myeloma cells compared to controls. ALT-803 treatment also significantly delayed paralysis and prolonged survival of BALB/c mice bearing MOPC-315P tumors with an increased frequency of dosing providing better responses. No toxicity was observed following treatment, indicating that ALT-803 administration and its anti-tumor effects, which resulted in the rapid killing of a large number of myeloma cells over a short duration, were well tolerated by mice. Overall, these results demonstrate that ALT-803 has substantial anti-tumor activity against established BM myeloma cells and that this activity is significantly more potent than that of IL-15 (7).

Since ALT-803 treatment was capable of essentially curing mice bearing 5T33P myeloma, it was possible to evaluate whether these mice retain immunological memory against the tumor cells. C57BL/6 mice that survived initial 5T33P inoculation due to ALT-803 treatment were not affected by 5T33P cell re-challenge 3 months later, even in the absence of additional ALT-803 administration. These mice continued to survive over 6 months from the initial tumor cell inoculation. In contrast, all of the treatment-naïve

mice administered 5T33P cells on the same study days subsequently exhibited paralysis with median survival of 29 days post tumor cell injection. These results demonstrate that a short course of ALT-803 treatment not only has potent efficacy against established 5T33P myeloma but also is capable of inducing long-lasting protective immunologic memory to subsequent tumor cell re-challenge (7).

As summarized above, ALT-803 is a novel IL-15 based protein complex that is structurally and functionally distinct from NCI rhIL-15 cytokine. The development and application of an ALT-803 regimen that both increases the level of activated T cells and NK cells to predictable levels and has an acceptable safety profile could have profound benefits when used as either a single agent or in combination with tumor vaccines, monoclonal antibodies, T-cell therapy, other immunotherapy agents such as anti-CTLA4 and anti-PD1, and targeted therapeutic agents. Despite the enthusiasm for the potential of the NCI rhIL-15, the ALT-803 presentation of IL-15Rα with the IL- 15N72D superagonist could be a more ideal formulation that will be more effective at lower concentrations. Its longer half-life due to fusion with Fc will mimic the effect of continuous infusion, but with a more practical regimen that can be combined with other therapies.

3.7.5. Comparative Studies of Intravenous and Subcutaneous ALT-803

Emerging data from ongoing trials using the NCI's recombinant human IL-15 (rhIL-15) product suggests that IV dosing likely is not optimal for IL-15 because it induces a high C_{max} and secondary cytokine release (IL-6 and IFNγ) that affects its tolerability therefore limiting. Alternately, based on pre-clinical studies, other early clinical studies using rhIL-15, and published studies with IL-2, sub-Q dosing is safer and provides much better tolerability. For example, Waldmann and colleagues conducted the first in human solid tumor trial of rhIL-15 using daily IV bolus infusion for 12 consecutive days (58). DLTs observed at the 3.0 and 1.0 μg/kg per day cohort were grade 3 hypotension, thrombocytopenia, and elevations of ALT and AST. The MTD was declared at 0.3 μg/kg per day. In contrast, the ongoing sub-Q trial using the same rhIL-15 product being conducted through the CITN (Protocol CITN11-02: A Phase 1 Study of Recombinant IL15 (rhIL15) in Adults with Advanced Solid Tumors: Melanoma, Renal Cell, Non-Small Cell Lung and Head and Neck Cancer – Dr. Jeffrey Miller – PI) has successfully completed dose cohorts of 0.25, 0.5, 1.0, and 2.0 μg/kg of rhIL-15 given as 10 daily doses out of 12 days (no dosing on days 6 and 7). The study is currently enrolling at 3 μg/kg with no dose limiting toxicity. The major conclusion from this experience is that going from IV to sub-Q dosing increases safety and allows for a MTD ≥ 6 times higher. The increased tolerance of sub-Q dosing is likely a result of a decreased C_{max} compared with the same dose level administered intravenously and more sustained levels of the rIL-15 product in circulation. Lowering the C_{max} allows for more drug delivery overall.
To extend these findings to ALT-803, preclinical studies were conducted to evaluate IV and sub-Q administration of ALT-803 in terms of the pharmacokinetics, immunostimulation, and antitumor efficacy in C57BL/6 mice. Initial studies of C57BL/6 mice treated with 0.2 mg/kg ALT-803 showed an estimated half-life of 5.3 hours for IV administration and 3.8 hours for sub-Q administration. The maximal serum concentration of ALT-803 was 650 ng/ml at the 20 hour time point following sub-Q administration and 1700 ng/ml at 2 hour time point following i.v. administration. In terms of immune stimulation, ALT-803 administered sub-Q or IV could equally induce

proliferation of $CD8^+$ T cells and NK cells. Additionally, IV and sub-Q administration of ALT-803 similarly activated immune cells to reduce tumor burden in the bone marrow of 5T33 myeloma-bearing mice. Both IV and sub-Q administration of ALT-803 at up to 0.2 mg/kg was well tolerated in normal and tumor-bearing C57BL/6 mice.

A follow-up pilot study for toxicological effects of 1 mg/kg ALT-803 injected sub-Q weekly for 4 weeks in C57BL/6 mice revealed immune system-related changes that were similar to those seen in a previous toxicology study in which the C57BL/6 mice were treated with the same ALT-803 dosing regimen using an IV route. No mortalities were observed in mice following 4 weekly sub-Q injections of 1 mg/kg ALT-803. With the exception of slight weight loss and the observance of hunched posture after the first sub-Q injection of ALT-803, no clinical signs of test article related toxicities were observed during this study. Examination of the peripheral blood revealed that there were increased numbers of WBC and lymphocyte counts compared to PBS controls. Overall, there was a 9-fold increase for both WBCs and lymphocytes in animals treated with ALT-803 compared to PBS injected mice. An increase in neutrophils, monocytes, eosinophils and basophils was also observed in sub-Q ALT-803 treated mice. Significant increases in the weight of spleen, lymph node, and liver (5.5, 3, and 1.3-fold respectively) of ALT-803 treated mice were observed. Comparable broad-based expansion of immune cells and increased weights of lymphoid organs was previously reported for mice receiving multidose IV treatment with ALT-803.

Overall, the results of the preclinical studies of ALT-803 indicated that sub-Q dosing decreases the C_{max} compared to IV dosing but retains the immunostimulatory activity and antitumor efficacy without exaggerating toxicity.

3.7.6. Activity of ALT-803 Combination Therapies in Tumor Efficacy Models

Since ALT-803 is capable of activating murine and human NK cells, studies were conducted to evaluate whether ALT-803 could augment rituximab (anti-CD20 mAb)-directed ADCC of human NK cells against B cell lymphomas and facilitate B lymphoma clearance *in-vivo*. Short-term *in-vitro* treatment of human PBMCs with ALT-803 was found to increase the expression of the cytotoxic effector proteins, granzyme B, and perforin in human NK cells and $CD8^+$ T lymphocytes. ALT-803 also potentiated rituximab-directed human PBMC ADCC against the $CD20^+$ human Daudi B lymphoma cell line *in vitro*. In order to investigate antitumor efficacy of ALT-803 plus rituximab treatment, Daudi cells were engrafted into NK cell-competent SCID mice. Tumor-bearing mice were then treated (days 15 and 18) with vehicle, rituximab, ALT-803, or ALT-803 plus rituximab. Daudi cell burden in the BM was assessed at day 22. Mice treated with ALT-803+rituximab had significantly reduced Daudi cell burden in BM, compared to rituximab, ALT-803, or vehicle treatment groups. The enhanced antitumor activity of the combination therapy was ALT-803 dose-dependent. Furthermore, tumor-bearing mice treated with ALT-803+rituximab had prolonged survival compared to ALT-803 or rituximab monotherapy groups. ALT-803 was well tolerated at all of the administered dose levels in combination with rituximab. Thus, ALT-803 represents an effective IL-15 superagonist that augments immune cell cytotoxic potential and ADCC against malignant B cells *in vitro*, and significantly increases rituximab-triggered clearance of B cell lymphoma by immune cells *in vivo*.

In addition to antibody-based combinations, ALT-803 has been evaluated with immunostimulatory BCG therapy. Intravesical BCG is standard-of-care for patients with

NMIBC. BCG has been shown to induce specific immunologic responses (i.e., local induction of IL-2 release and activation of effector T-cell responses) that correlate with clinical benefit to patients (23-27). Based on these results, studies were conducted to evaluate whether intravesical administration of ALT-803 along with BCG could generate an enhanced immunologic response leading to significant bladder tumor burden reduction. Using a well-established carcinogen induced rat NMIBC model, the efficacy of intravesical ALT-803 with and without BCG was assessed. Rat tissues were evaluated to document treatment response. Intravesical ALT-803 was safe and well tolerated alone and in combination with BCG. As a single treatment agent, ALT-803 reduced tumor burden by 35% compared to control, whereas BCG alone only reduced tumor burden by 15%. However, the combination of ALT-803 plus BCG reduced tumor burden by 46% compared to control. Immune monitoring suggested that the antitumor response was linked to the production and secretion of IL-1α, IL-1β and RANTES, which in turn, induced the proliferation and activation of NK cells. Lastly, tumoral responses of the combinational treatment were associated with 76% reduction in angiogenesis, which is significantly higher than when assessed with either agent alone. The enhanced therapeutic index seen with the ALT-803+BCG duplet provided justification for the development of this regimen for clinical evaluation (28).

Further studies of ALT-803 in combination with checkpoint blockade (i.e., anti-PD-1, anti-PD-L1, and anti-CTLA-4 mAbs) were conducted in various solid and hematologic tumor models as described below.

3.8. PRE- CLINICAL COMBINATION OF CHECKPOINT BLOCKADE THERAPY WITH IL-15- OR ALT-803

While PD-1/PD-L1 pathway or IL-15/IL-15Ra complexes have considerable efficacy as single therapies, as outlined above, their greatest potential may be as combinatorial therapies. The rationale for this combinatorial therapy comes from a number of studies both published and unpublished, which are summarized below.

Three published preclinical studies that warrant mentioning used recombinant IL-2 or IL-15 cytokines. First, West et al. treated mice with chronic viral (LCMV) infection using a combination of IL-2 and anti-PD-1 mAb (69). Both these agents are effective as singular therapy in this model. However, together, there was a synergistic ability to augment the expansion of viral-reactive T cells as well as reduce LCMV titers. Furthermore, the authors demonstrated that exhausted viral-specific T cells recovered their functional ability with the combinatorial therapy, and thus the responding T cells were reinvigorated. Given the similarities between T cell exhaustion in chronic viral infection and T cell dysfunction in tumor progression (70,71), these findings suggest that a combination of cytokine therapy and checkpoint blockade would be an effective strategy in enhancing responses in cancer patients.

Other preclinical studies conducted in Waldmann's laboratory assessed IL-15 and anti-PD-L1 mAb and/or anti-CTLA4 mAb in mouse models of colon and prostate cancer. Yu et al. demonstrated that the combination of IL-15 and anti-PD-L1 mAb was more effective than either agent alone in reducing the number of lung metastases, as well as improving overall survival in mice bearing CT-26 colon tumor cells (72). In a second study, Yu et al. evaluated IL-15 combined with anti-PD-L1 and anti-CTLA4 mAb in the established mouse TRAMP-

C2 prostate tumor model. IL-15 treatment resulted in a significant prolongation of survival in tumor-bearing animals. Co-administration of either anti-PDL1 or anti-CTLA-4 mAb with IL-15 did not improve animal survival over that of IL-15 alone; however, treatment with the combination of IL-15 plus both anti-CTLA-4 and anti-PD-L1 mAbs was associated with decreased rates of tumor growth and improved animal survival. An increase in tumor-specific CD8 T cell responses and lower Treg activity was also observed in this combination as compared with IL-15 alone. The authors concluded that combining the immune stimulatory properties of IL-15 with simultaneous removal of 2 critical immune inhibitory checkpoints resulted in enhancement of immune responses, leading to increased antitumor activity (73).

3.8.1. Activity of ALT-803 in Combination with Checkpoint Blockade in Tumor Efficacy Models

The anti-tumor activity of combination therapy of ALT-803 with checkpoint inhibitors has been evaluated in the tumor models where ALT-803 monotherapy showed efficacy. In the C57BL/6 mice bearing 5T33P myeloma tumors, treatment with either ALT-803 at 0.2 mg/kg or anti-PD-L1 mAb at 5 mg/kg (2 weekly doses) were capable of increasing animal survival but lower dose levels were not effective. However, combination therapy of suboptimal ALT-803 (0.05 mg/kg) plus anti-PD-L1 Ab (0.25 mg/kg) was significantly more effective at prolonging survival of 5T33P tumor-bearing mice than either monotherapy, demonstrating synergistic anti-tumor activity of ALT-803 in combination with PD-L1 checkpoint blockade (Fig. H).

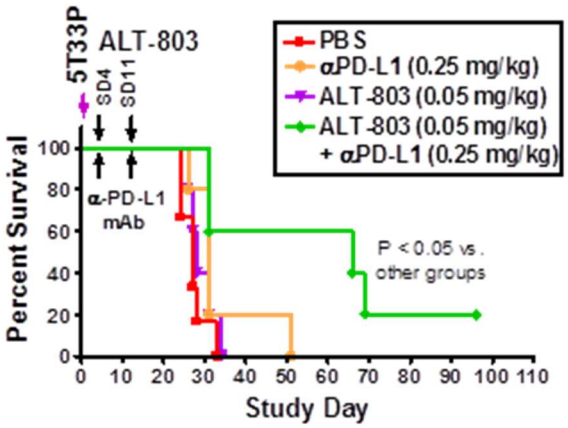


Figure H. Effect of ALT-803 plus anti-PD-L1 mAb therapy on survival 5T33P bearing mice. C57BL/6 mice (5/group) were injected IV with PD-L1-positive 5T33P multiple myeloma cells (10^7/mouse) and then treated IV 4 and 11 days later with PBS, anti-PD-L1 Ab, ALT-803 or combination therapies at the indicated concentrations. Animal survival (using hind leg paralysis as an endpoint) was assessed and Kaplan-Meier survival curves are shown.

In the CT-26 colon carcinoma metastasis model, combination therapy using ALT-803 plus anti-PD-L1 and anti-CTLA4 mAbs was also more effective than ALT-803 monotherapy in prolonging survival of tumor-bearing mice (Fig. I). Consistent with improved activity of ALT-803, the ALT-803-based combination therapy was significantly more active in this model than IL-15 plus anti-PD-L1 and anti-CTLA4 mAbs, even when IL-15 was administered at a >10-fold higher cumulative dose.

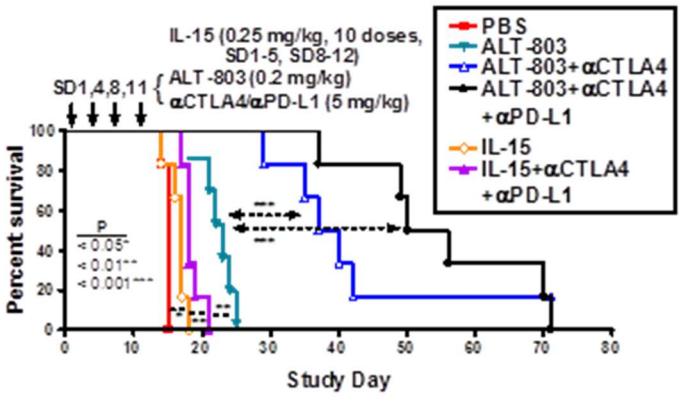


Figure I. Effect of ALT-803 plus anti-PD-L1 and anti-CTLA4 mAb therapy on survival CT-26 bearing mice. Survival curves of BALB/c mice injected IV with CT26 colon carcinoma cells and subsequently treated with IV PBS (control), ALT-803 or IL-15 monotherapy, or ALT-803 or IL-15 combination therapy with checkpoint inhibitors as indicated.

Extensive studies of ALT-803 monotherapy and in combination with anti-PD-1 mAb were also carried out in C57BL/6 mice that had been intracranially implanted with the glioblastoma cell line, GL261-luc. Treatment with multiple doses of ALT-803 (3 or 4 doses) or anti-PD-1 mAb (3 doses) as monotherapy starting 7 to 10 day post-tumor implantation exhibited similar increases in antitumor activity and prolonged animal survival when compared to PBS-treated controls (Fig. J). The combination of ALT-803 and anti-PD1 mAb treatment further extended median survival times of tumor-bearing mice. It was also found that anti-PD-1 mAb in combination with 4 doses of ALT-803 increased the percentage of long-term tumor free survivors (>60 days post-implantation) to 40% from the 20% rate observed in mice treated with anti-PD-1 Ab and ALT-803 monotherapy. Interestingly, the "cured" mice were resistant to tumor rechallenge, suggesting treatment-induced immune memory response against the tumor. These results suggest that combining the immunostimulatory activity of ALT-803 with the checkpoint blocker, anti-PD-1 Ab, has a beneficial additive effect in prolonging survival of glioblastoma tumor bearing mice.

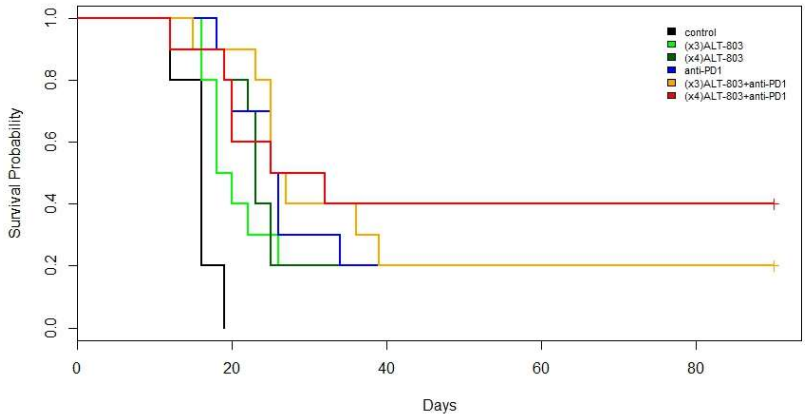


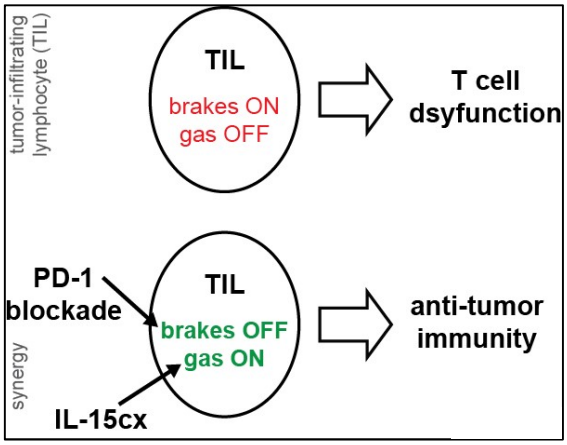
Figure J. Effect of ALT-803 plus anti-PD-1 mAb on survival GL261-luc glioblastoma bearing mice. Survival curves of C57BL/6 mice implanted intracranially with GL261-luc glioblastoma cells and subsequently treated with IV PBS (control), ALT-803 or ALT-803 + anti-PD-1 mAb as indicated. ALT-803 (3 doses) + anti-PD-1 Ab vs. PBS, P=0.001; ALT-803 (4 doses) + anti-PD-1 Ab vs. PBS, P=0.002

Cumulatively, these pre-clinical results provide compelling data that the combination of ALT-803 and checkpoint blockade therapy may be an effective treatment for patients with metastatic cancer. Below, we review the clinical data that support this hypothesis.

3.9. RATIONALE AND HYPOTHESIS FOR COMBINING NIVOLUMAB AND ALT-803 FOR METASTATIC NSCLC

Given the above background, we hypothesize that combined checkpoint blockade inhibition and appropriate cytokine therapy will lead to synergistic outcomes in the induction of anti-tumor immune responses in the presence of dysfunctional tumor-reactive lymphocytes. To envision this synergy, one might consider a broken automobile where the brake pedal is stuck down and there is no gasoline (Fig. K). If we cut the brake pedal line, the car will no longer be stuck, much like blockade of inhibitory pathways in patients (i.e. PD-1 blockade). However, without gas, the car will have limited mobility, or without cytokine, T cells may not be able to deliver a sustained anti-tumor response. We propose that effective combinatorial therapy will be like providing gasoline (addition of IL-15 superagonist complex, ALT-803) after the brake pedal line is cut, and thus, revived T cells will function effectively upon cytokine stimulation.

Figure K.



3.10. SAFETY AND BIOLOGICAL EFFECTS OF ALT-803 IN HUMANS

Five early stage trials are currently being conducted using ALT-803 in patients with various cancer indications. The following clinical study summaries are current as of July 2015.

The first trial is an Altor-sponsored multi-center clinical study of ALT-803 underway under IND 118280 in patients with metastatic melanoma. The study is being conducted as a dose escalation with one patient each to be enrolled in the first two cohorts and a minimum of three patients to be enrolled in the last three cohorts to determine the MTD or OBD of ALT-803. Enrolled patients receive two 6-week cycles consisting of 4 weekly ALT-803 intravenous doses followed by a 2-week rest period. Patients with stable or benefitting disease will be eligible to receive up to two additional 6-week cycles. One patient enrolled to the 0.3 µg/kg ALT-803 dose level. The reported study drug-attributed adverse events were transient low-grade fever, rigors, nausea and vomiting for the first dose with no pre-medications. For the second through fifth doses, the patient was pre-medicated with acetaminophen and the rest of the reported AEs were mild to moderate. The next patient enrolled to the 0.5 µg/kg dose level. The patient was pre-medicated with acetaminophen and received the first dose with no side effects. All reported AEs were mild to moderate including nausea, fatigue and pruritus. Three patients enrolled and completed the study treatment at the 1 µg/kg ALT-803 dose level. All AEs reported for these patients were mild to moderate, including chills, worsening constipation and

hypertension. The study protocol for this trial was recently amended to include renal cell carcinoma, non-small cell lung carcinoma and squamous cell head and neck carcinoma. Three patients, including one patient with renal cell carcinoma, have enrolled and completed treatment at the 3 μg/kg dose level. AEs reported so far were mild to moderate including fever, fatigue, vomiting and myalgia. Two patients, including one patient with renal cell carcinoma, have enrolled at the 6 μg/kg dose level. Data collection on adverse events is ongoing.

The second trial is an investigator-sponsored multi-center clinical study of ALT-803 underway under IND 119578 in patients with hematologic malignancy who have relapsed after ASCT. The first phase of this study is being conducted under a standard 3+3 design of dose escalation for toxicity. Enrolled patients receive ALT-803 intravenous doses given once weekly for 4 weeks. Six patients have enrolled and completed the study treatment at the 1 μg/kg ALT-803 dose level. For the first three patients, the reported study drug-attributed AEs were fever, chills, rigors and edema. Grade 1 fever in two patients occurred approximately 4 to 5 hours after ALT-803 dosing and then subsided approximately 6 to 7 hours after ALT-803 dosing. Grade 2 rigors occurred in two patients, grade 2 chills occurred in two patients and grade 1 chills occurred in one patient. Grade 2 rigors in one patient required Demerol for 3 out of 4 ALT-803 doses. One patient experienced grade 2 edema and another patient experienced grade 1 edema. The first three patients experienced asymptomatic hypotension (BP = 80/56, 86/68 and 83/60, respectively) but the patients were normotensive after fluid administration without the recurrence of hypotension episodes. None of the treated patients were pre-hydrated with fluids. Grade 2 skin rash was also observed in one patient after the second dose of ALT-803, which was consistent with graft-versus-host disease (this did not require systemic therapy). The fourth, fifth and sixth patients received the study treatment with no reported AEs. Three patients completed the study treatment at the 3 μg/kg ALT-803 dose level. The patients received hydration prior to each dose, reported AEs include grade 1 fever and chills 6 and 10 hours after ALT-803 dosing. Three patients enrolled and completed treatment at the 6 μg/kg ALT-803 dose level. Most common reported AEs in this cohort include mild to moderate fever, rigors and flu-like symptoms. Two patients completed treatment and one patient is being treated at the 10 μg/kg ALT-803 dose level. Reported AEs for the first patient after the first dose of ALT-803 include transient fever, nausea and vomiting. The AEs started around 3 hours after dosing and lasted approximately 4 hours. The patient also experienced low grade asymptomatic hypotension with systolic blood pressure in the 80's. IV fluids were administered and the blood pressure returned to baseline. The second patient experienced transient fever and chills after the first dose of ALT-803. The chills were controlled with Demoral. The patient did not experience hypotension. Additional AE data will be reported as it becomes available. The protocol was recently amended to change the administration of ALT-803 from IV to subcutaneous injection starting at the 6 μg/kg dose level. Patient enrollment is complete at the 10 μg/kg dose level with IV administration of ALT-803. Patient enrollment for subcutaneous injection at 6 μg/kg will be initiated once IRB approval is confirmed.

Clinical biomarker assessment is being conducted. For the study of patients with hematologic malignancy after ASCT, preliminary data is available on the Ki-67 analysis of NK, $CD4^+$, $CD8^+$ and NKT cell subsets and serum cytokines of the patients' pre-dose and post-dose specimens. Both serum level of IFN-γ and IL-6 were induced in a dose-dependent manner

within the dose range from 1 μg/kg to 6 μg/kg. $Ki67^+$ NK, $CD8^+$ and $CD4^+$ T cells increased after ALT-803 dosing at a dose level of ≥3 μg/kg in all patients. Thus, the preliminary data suggests that ALT-803 consistently promotes the activation and proliferation of NK, T and NKT cells for certain patients at a dose level of ≥ 3μg/kg with this indication. Preliminary PK data from the first 11 patients treated with 1 μg/kg, 3 μg/kg and 6 μg/kg of ALT-803 indicated a serum half-life of approximately 3 hours and a maximum concentration of about 45, 485 and 1280 pM, respectively. Additional information on the pharmacokinetic profile of ALT-803 in humans will be provided as it becomes available.

The third trial is an Altor-sponsored multi-center clinical study of ALT-803 underway under IND 114006 in patients with relapsed or refractory multiple myeloma. The first phase of this study includes a classic (3+3) dose escalation to determine the MTD or MED and to designate a dose level for the phase II two-stage expansion. The dose levels are 1, 3, 6, 10 and 20 μg/kg of ALT-803. Enrolled patients will receive two 6-week cycles consisting of 4 weekly ALT-803 intravenous doses followed by a 2-week rest period. Patients with stable or benefitting disease will be eligible to receive up to two additional 6-week cycles. Three patients enrolled and completed the study treatment at the 1 μg/kg ALT-803 dose level. All AEs reported for these patients were mild to moderate, including constipation, nausea, fatigue, ALC decreased and WBC count decreased. All patients are receiving pre-medications. On patient completed treatment and two patients are undergoing treatment at the 3 μg/kg ALT-803 dose level. Reported AEs include mild to moderate fevers, rigors and neutropenia. Additional AE data will be reported as it becomes available.

The fourth trial is an Altor-sponsored multi-center clinical study of ALT-803 in combination with BCG underway under IND 121976 in patients with BCG-naïve non-muscle invasive bladder cancer. The first phase of this study includes a classic (3+3) dose escalation to determine the MTD) of ALT-803 and to determine the RD of ALT-803 combined with BCG for the stage II expansion phase. The dose levels are 100, 200 and 400 μg/instillation of ALT-803 plus standard BCG (50 mg/instillation). The expansion phase consists of a noncomparative randomized design of patients receiving ALT-803 at the RD level in combination with BCG or BCG alone. Enrolled patients will receive BCG plus ALT-803 weekly via a urinary catheter in the bladder for 6 consecutive weeks. Three patients enrolled and completed treatment in the first cohort of 100 μg/instillation of ALT-803 plus BCG. Reported study drug-attributed adverse events included mild nausea, headache, hematuria and urinary tract pain and moderate cystitis noninfective. Three patients have enrolled and completed treatment in the 200 μg/instillation of ALT-803 plus BCG. Reported study drug-attributed adverse events included mild hematuria and urinary incontinence. Two patients completed treatment and one patient is undergoing treatment in the 400 μg/instillation of ALT-803 cohort. AE data will be reported as it becomes available.

The fifth trial is an Altor-sponsored multi-center clinical study of ALT-803 plus rituximab underway under IND 114006 in patients with relapsed or refractory indolent B cell non-Hodgkin's lymphoma. The first phase of this study includes a classic (3+3) dose escalation to determine the MTD or MED and to designate a dose level for the phase II two-stage expansion. The dose levels are 1, 3 and 6 μg/kg of ALT-803. Enrolled patients will receive a 4-week induction cycle consisting of 4 weekly doses of ALT-803 and standard rituximab (375 mg/m^2) by intravenous injection, respectively. Patients with stable or benefitting disease will be eligible to receive up to four consolidation treatment cycles consisting of a single treatment

of ALT-803 plus rituximab, repeated every 8 weeks for a total of 4 additional ALT-803 and rituximab doses. Three patients enrolled and are currently undergoing treatment at the 1 µg/kg ALT-803 dose level. AEs reported thus far for this patient were mild to moderate including edema, ALC decreased and WBC count decreased. Additional AE data will be reported as it becomes available.

Additional results of these studies, including serum cytokine levels, pharmacokinetic and immunogenicity analysis and tumor response assessment, will be reported as it becomes available.

3.11. JUSTIFICATION FOR ALT-803 DOSING

For the dose escalation study of patients with advanced cancer, consideration for a starting dose of ALT-803 have been based on the desire to provide immunostimulatory activity without anticipated toxicity. The principals described in the ICH guidance: ICH S6 and S6 (R1) "Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals", ICH S9 "Nonclinical Evaluation for Anticancer Pharmaceuticals", and the FDA guidance entitled "Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers" provide guidance on establishing such a starting dose level. Cell binding studies demonstrated that immune cells from C57BL/6 mice and cynomolgus monkeys were capable of binding ALT-803 with similar affinities as human immune cells. Thus, these animal species are relevant for assessing the toxicity and pharmacodynamics of ALT-803. Based on allometric scaling to an equivalent human dose, the activity and toxicity profiles of ALT-803 at 0.1 mg/kg in mice and 0.03 mg/kg in cynomolgus monkeys indicate that the starting dose of 0.01 mg/kg in humans should provide a minimally anticipated biologic effect level. This is further supported by the nonclinical studies that ALT-803 at the relevant dose levels does not trigger a broad-based cytokine storm by either human or murine immune cells. The non-clinical studies also support a weekly dosing regimen of ALT-803 as one to provide biological activity and anti-tumor efficacy without cumulative or irreversible toxicities. Based on the results of these studies, Altor initiated the investigator-sponsored study of ALT-803 in patients with hematologic malignancy who relapsed after ASCT using 1 µg/kg as the initial dosing level as described above (Section 3.10). None of the treated patients, including the patients dosed at the 10 µg/kg ALT-803 dose level, experienced DLT. Therefore, Altor proposes to use 6 µg/kg as the starting dose level for ALT-803 administered weekly for five weeks per cycle in combination with nivolumab in patients with relapsed or refractory squamous NSCLC. Patients in the proposed study will be closely monitored for lymphocytosis and cytokine-induced toxicities.

Over 100 patients have been treated with ALT-803 as a single agent or in combination with BCG, rituximab and nivolumab; ALT-803 been administered intravescicularly, intravenously, and subcutaneously, and has been administered in doses ranging from 0.1 mcg/kg to 20 mcg/kg. This trial has escalated the latter agent treating 3 patients at 6 mcg/kg, 3 patients at 10 mcg/kg, and 5 patients at 15 mcg/kg. If no DLTs are observed at the 15mcg/kg dose level, this study will continue to escalate to a dose of 20 mcg/kg as the study has observed no dose limiting toxicities with easily manageable outpatient subcutaneous administration.

4. ELIGIBLITY CRITERIA

4.1. INCLUSION CRITERIA

1. Histologically or cytologically confirmed diagnosis of NSCLC who present with Stage IIIB/Stage IV disease (according to version 7 of the International Association for the Study of Lung Cancer Staging Manual in Thoracic Oncology), or recurrent disease following radiation therapy or surgical resection.
2. Patient must be eligible for treatment with nivolumab. Patients previously treated with nivolumab, pembolizumab or atezolizumab and who have progressed are eligible.

   Patients with targetable with EGFR or ALK mutations are eligible after disease recurrence or progression after at least one targeted therapy for advanced or metastatic disease. EGFR or ALK mutated patients are not required to have received platinum-based doublet chemotherapy prior to enrollment on this trial.

3. Measurable disease as defined by RECIST 1.1 criteria.
4. Age ≥ 18 years
5. Performance status: ECOG performance status of ≤1 (Appendix A)
6. Adequate organ system function within 14 days of registration:

ANC	> 750/µL (>0.75 X 10^9/L)
PLT	≥ 100,000/µL (≥ 30 X 10^9/L)
HGB	≥ 8g/dL
Total bilirubin	≤ 2.0 x ULN
AST	≤ 3.0 X ULN
ALT	≤ 3.0 X ULN
eGFR*	> 45mL/min

**using Cockcroft & Gault equation (see Appendix B)*

7. Negative serum pregnancy test if WOCBP (non-childbearing is defined as greater than one year postmenopausal or surgically sterilized).
8. Female participants of childbearing potential must adhere to using a medically accepted method of birth control up to 28 days prior to screening and agree to continue its use during the study or be surgically sterilized (e.g., hysterectomy or tubal ligation) and males must agree to use barrier methods of birth control while on study. WOCBP must agree to use effective contraception during treatment and for at least 5 months following the last dose of study treatment.
9. Prior to any study specific activities, the patient must be aware of the nature of his/her disease and willingly consent to the study after being informed of study procedures, the experimental therapy, possible alternatives, risks and potential benefits.

4.2. EXCLUSION CRITERIA

1. While prior therapy with nivolumab, pembrolizumab, or atezolizumab is allowed,

any prior therapy with other anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, anti-CTLA-4 antibody (including ipilimumab or any other antibody or drug specifically targeting T-cell co-stimulation or checkpoint pathways) is not allowed.

2. NYHA Class III or IV heart failure (Appendix C), uncontrollable supraventricular arrhythmias, any history of a ventricular arrhythmia, or other clinical signs of severe cardiac dysfunction.
3. Symptomatic congestive heart failure, unstable angina pectoris, or myocardial infarction within 6 months of registration.
4. Marked baseline prolongation of QT/QTc interval (e.g. demonstration of a QTc interval greater than 500 milliseconds).
5. Patients with CNS metastases with the following exceptions: Patient untreated CNS metastases with 5 or fewer sites of disease, with no single site larger than 20mm, are eligible if they are asymptomatic and not requiring steroids at any dose. Patients with asymptomatic CNS metastases may be treated with radiosurgery before or during therapy on trial without treatment delays. Patients with treated, symptomatic CNS metastases are eligible if they are neurologically returned to baseline (except for residual signs or symptoms related to the CNS treatment) for at least 2 weeks prior to registration AND either off corticosteroids, or on a stable or decreasing dose of ≤ 10 mg daily prednisone (or equivalent).
6. Known autoimmune disease requiring active treatment. Subjects with a condition requiring systemic treatment with either corticosteroids (>10 mg daily prednisone equivalent) or other immunosuppressive medications within 14 days of registration are excluded. Inhaled or topical steroids, and adrenal replacement steroid doses ≤ 10 mg daily prednisone equivalent, are permitted in the absence of active autoimmune disease.
7. Subjects with a history of interstitial lung disease and/or pneumonitis.
8. Known HIV-positive.
9. Active systemic infection requiring parenteral antibiotic therapy. All prior infections must have resolved following optimal therapy.
10. Positive hepatitis C serology or active hepatitis B infection. Chronic asymptomatic viral hepatitis is allowed.
11. Women who are pregnant or nursing.
12. Psychiatric illness/social situations that would limit compliance with study requirements.
13. Any ongoing toxicity from prior anti-cancer treatment that, in the judgment of the investigator, may interfere with study treatment. All toxicities attributed to prior anti-cancer therapy other than alopecia and fatigue must resolve to grade 1 (NCI CTCAE version 4) or baseline prior to registration.
14. Anti-cancer treatment including surgery, radiotherapy, chemotherapy, other immunotherapy, or investigational therapy within 14 days of registration.
15. Other illness that in the opinion of the investigator would exclude the patient from

participating in this study, including uncontrolled diabetes mellitus, cardiac disease or decreased pulmonary function.

4.3. PHASE II ARM ASSIGNMENT

- Arm A will be comprised of patient not previously treated with nivolumab, pembrolizumab or atezolizumab.
- Arm B will be comprised of patient who have been treated and progressed on nivolumab, pembrolizumab or atezolizumab.

4.4. INCLUSION OF WOMEN AND MINORITIES

Both men and women of any races and ethnic groups are eligible for this treatment study. There is no evidence thus far for differing treatment outcomes based on gender or ethnicity.

4.5. PATIENT REGISTRATION

The MUSC HCC SIS Unit will provide patient registration services for the study. The SIS Unit will conduct a patient eligibility audit review of all eligibility source documents prior to patient registration. These procedures are outlined in the MUSC 102323 Operations Manual. After obtaining signed informed consent and completion of required baseline assessments, eligible subjects will be registered upon the SIS-Unit's verification of study eligibility. A unique subject number will be assigned to each patient. The SIS Unit will issue a patient registration confirmation email to the enrolling study team at the time of registration. This confirmation will include the patient's assigned study number, cohort dose level and arm assignment (for Phase II).

5. TREATMENT

5.1. AGENT ADMINISTRATION

Cohorts will be treated with escalating dose of ALT-803 and nivolumab. Nivolumab will be administered on Day 1 of every other week of each cycle beginning Week 1 Day 1 and continue until disease progression as defined in Section 11 or unacceptable toxicity. ALT-803 will be administered over four 6-week cycles consisting of treatment on Day 1 of Weeks 1-5 followed by one week (W6) of rest.

The study drug ALT-803 will be administered subcutaneously. Injection sites should be rotated per institutional guidelines and each injection site separated by at least 1 inch. Nivolumab will be administered as described in the product package insert provided by the manufacturer. On treatment days that nivolumab and ALT-803 are both administered, nivolumab will be administered first.

REGIMEN DESCRIPTION			
Agent	**Dose**	**Route**	**Schedule**
Nivolumab	240 mg	IV over 60 minutes[a]	Day 1 of Week 1, Week 3 and Week 5 of each cycle until progression
ALT-803	See Dose Escalation Schedule	sub-Q	Cycles 1-4: D1 of W1-5 W6 - Rest
a. +/- 15 minutes			

Treatment will start at dose level 1, and escalate in accordance with the dose escalation schedule below. A TiTE-CRM design will be used to guide dose escalation (see Section 10 for more details). The phase Ib portion of the trial will enroll up to 21 patients. The recommended phase II dose (RP2D) combination determined by the phase Ib portion of the study will be used to treat patients in the phase II portion of the study.

DOSE ESCALATION SCHEDULE		
Cohort	**ALT-803**	**Nivolumab**
-1	3 µg/kg	240 mg
1 (starting dose)	6 µg/kg	240 mg
2	10 µg/kg	240 mg
3	15 µg/kg	240 mg
4	20 µg/kg	240 mg

5.2. DEFINITION OF DOSE-LIMITING TOXICITY

The definitions of Dose Limiting Toxicity are described within this section and are categorized as Non-Immune-Related Adverse Events and Immune-Related Adverse Events. The DLT period is the first 28 days of treatment with ALT-803.

5.2.1. Non-Immune-Related Adverse Events

Non-Immune-Related Adverse Events are DLTs and are defined as follows: during the first 28 days of the study treatment, any toxicity that is not clearly unrelated to study treatment administration that is of Grade 3 and does not resolve to Grade 1 or lower within a week despite the use of medical intervention, or that is of Grade 4, but with exceptions as follows:

- The following events occurring during the DLT period **ARE** also considered a DLT.
    - Arrhythmia: Grade > 3 arrhythmia of any kind is a DLT.
    - ALC ≥ 50,000/μL sustained for 14 days is a DLT.
    - WBC ≥ 60,000/μL sustained for 14 days is a DLT.
    - Hypotension: Grade 3 that persists for > 4 hours **and** requires extended observation or hospitalization

- The following events occurring during the DLT period **ARE NOT** considered a DLT.
    - Any Grade 3 or 4 lymphopenia, leukopenia and/or neutropenia that recovers within 14 days is not a DLT.
    - Any Grade 3 or 4 thrombocytopenia with or without bleeding or anemia that recovers within 14 days is not a DLT.
    - Nausea or vomiting controllable with anti-emetics within 72 hours is not a DLT.
    - Hypotension (systolic pressure < 90 mm Hg) of Grade < 3 that is of limited duration (less than 72 hours) or can be managed with hydration measures as described in Section 6 is not a DLT.
    - Hypotension that requires a precautionary admission for observation after grade 3 hypotension that persists for ≤ 4 hours is not a DLT.
    - Grade 3 amylase and lipase elevations that recover within 14 days is not a DLT.
    - ALC > 20,000/μL but less than 50,000/ μL is not a DLT.

5.2.2. Immune-Related Adverse Events

The following Immune-Related Adverse Events are DLTs:

- Any Grade 4 irAE regardless of duration
- Any ≥ Grade 4 colitis regardless of duration
- Any Grade 3 or Grade 4 non-infectious pneumonitis regardless of duration
- Any Grade 3 irAE, excluding colitis and pneumonitis, that does not downgrade to ≤ Grade 2 within 3 days after onset of the event despite maximal supportive care including systemic corticosteroids or downgrade to ≤ Grade 1 or baseline within 14 days

- Any Grade 2 pneumonitis that does not downgrade to ≤ Grade 1 within 3 days of the initiation of maximal supportive care
- Liver transaminase elevation higher than 8 × ULN or total bilirubin higher than 6 × ULN
- Any other toxicity that is greater than baseline grade, is clinically significant and/or unacceptable, and is judged to be a DLT by the Sponsor-Investigator

The definition *excludes* the following conditions:

- Grade 3 endocrinopathy that is managed with or without systemic corticosteroid therapy and/or hormone replacement therapy and the subject is asymptomatic
- Grade 3 inflammatory reaction attributed to a local antitumor response (eg, inflammatory reaction at sites of metastatic disease, lymph nodes, etc) that resolves to ≤ Grade 1 within 4 weeks
- Concurrent vitiligo or alopecia of any AE grade

Management and dose modifications associated with the above adverse events are outlined in Section 6.

5.3. PRE AND POST THERAPY INTERVENTION GUIDELINES

The ALT-803 intervention guidelines described in this section should be followed, for the first cycle of drug administration. After the first cycle, these guidelines may be modified by the individual study center as medically necessary or as appropriate without requiring a protocol amendment or being considered a protocol deviation. Standard treatment of nivolumab infusion reactions is appropriate, with sparing use of steroids whenever possible.

Condition	Agents	Dose	Route	When
Fever/chills	Acetaminophen	up to 650 mg or 10 to 15 mg/kg recipient weight	Orally	Prior to each dose & repeat 4 hours after dosing. Repeat every 4 hours if fever present
	AND			
	Indomethacin	25 mg daily dose or 50 mg when patient experienced a fever > 39.0°C in a previous dose	Orally	Prior to each dose & repeat it 4-6 hours after dosing If persistent fever > 39.0°C, repeat 50 mg every 8 hours (if adequate renal function) with acetaminophen every 6 hours.
Allergies	Diphenhydramine	25 mg or up to 0.5 to 1 mg/kg recipient weight	Orally or IV	One hour prior to each dose and Repeat 4-6 hours after dosing. Antihistamine, such as diphenhydramine or cetirizine, should

Condition	Agents	Dose	Route	When
	In combination with one of the following:			be taken for one day before and one to two days after dosing.
	Ranitidine	300 mg	Orally	
	Cimetidine	300 mg	Orally	
	Famotidine	20 mg	Orally	
Hypotension	Normal saline (0.9%)	2 mL/kg/hr (up to 100 mL/hr)***	Continuous IV	Begin 30 min prior to and for 6 hours after each dose
Nausea/ Vomiting	Ondansetron	0.2 mg/kg (up to 8 mg)	IV	30 min prior to each dose
	OR other 5-HT3 antagonists at the discretion of the Investigator.			
Pain	Hydromorphone	0.5 mg	IV	30 min prior to each dose
	OR other opioid medications at the discretion of the Investigator			

***In the last 2 hours, if BP does drop slightly, it is recommended to increase the normal saline rate to 250 mL/hr (given no evidence of fluid overload) and give over 2 hours until pressure is 90 mm systolic or more stable. Alternatively, if this has occurred previously, a higher rate of fluid administration from the start of the dosing protocol (i.e. 200 mL/hr over 6 hours) can be used as long as there are no signs of fluid overload (i.e. crackles in the lungs or peripheral edema or >10% weight gain).

Note 1: Non-steroidal anti-inflammatory medication including acetaminophen, ibuprofen, or naproxen may be given per physician discretion following the recommended dosing thresholds:

Acetaminophen: not to exceed 3000 mg (3 grams) in 24 hours

Ibuprofen: not to exceed 2400 mg in 24 hours

Naproxen: not to exceed 1100 mg in 24 hours

Note 2: The use of systemic steroid medications may result in loss of therapeutic effects of the study drug and should be avoided; however in the event of a life-threatening inflammatory reaction to ALT-803, the IV administration of dexamethasone or other steroid-based medication is warranted.

5.3.1. ALT-803 Observation Requirements

For the first dose of ALT-803 on study, patients enrolled within the Phase Ib portion of the study will remain in the treatment center and be closely monitored for immediate adverse events for 6 hours. For each subsequent dose, patients will be monitored for up to 6 hours at the discretion of the investigator.

All patients enrolled within the Phase II portion of the study will remain in the treatment center and be closely monitored for immediate adverse events for a minimum of 2 hours and up to 6 hours after the first dose of ALT-803 at the discretion of the treating investigator. After the first dose, patients may be monitored for up to 6 hours at the discretion of the investigator. The required observation period for the Phase II portion of the study may be adjusted based on adverse reactions observed in the Phase Ib portion of the study.

During Post ALT-803 observation, vital signs (body temperature, heart rate, respiratory rate, systolic and diastolic blood pressure) will be documented at the following frequency until discharge from the clinic:

- Prior to each ALT-803 dose
- Post 15 minutes (+/- 5 min)

- Post 30 minutes (+/- 5 min)
- Post 60 minutes (+/- 10 min), and
- Post 120 minutes (+/- 15 min) minutes, then
- Hourly (+/- 15 min) post dose until discharge from the clinic or completion of dose monitoring.

5.3.2. Nivolumab Observation Requirements

Patient monitoring following nivolumab treatment will follow established standard of care.

5.4. SUPPORTIVE CARE GUIDELINES

The intervention guidelines described in this section can be modified by the individual study center as medically necessary or as appropriate without requiring a protocol amendment or being considered a protocol deviation.

Throughout the study, investigators may prescribe any concomitant medications or treatments deemed necessary to provide adequate supportive care. During the study, supportive therapy can include antibiotics, analgesics, pain control (transfusions, psychotherapy, growth factors, or any other symptomatic therapy as clinically indicated.

The ALT-803 protein complex comprises a variant of the IL-15 molecule, which is a common γ chain cytokine and shares the IL-2 receptor β and γ chains for signaling. Thus, ALT-803 may have similar immunostimulatory properties as IL-2. ALT-803 treatment, particularly at high-dose levels, could potentially cause similar side effects as commercially available IL-2, Proleukin® (Prometheus Laboratories Inc, San Diego CA) that is approved for use at high doses in advanced melanoma and advanced renal cell carcinoma. Minimal data is available to show the safety and effectiveness of ALT-803. Therefore, the supportive care guidelines for ALT-803 treatment described below and the drug interactions described in Section 8 are mainly modeled after the therapy guidelines for Proleukin®. For additional information of managing IL-2 induced toxicities, the Principal Investigator may refer to the approved product package insert for *Proleukin® (aldesleukin) for Injection*, *Proleukin® (aldesleukin) for Injection: High-Dose Guidelines* at the Proleukin® website and other published literatures reporting IL-2 therapy management guidelines.

5.4.1. Hypotension and CLS

CLS may begin immediately after treatment starts and may be marked by increased capillary permeability to protein and fluids and reduces vascular tone. In most patients, this will results in a concomitant drop in mean arterial blood pressure within 2 to 12 hours after the start of treatment. With continued treatment, clinically significant hypotension (defined as systolic blood pressure below 90 mm Hg or a 20 mm Hg drop from baseline systolic pressure) and hypoperfusion may occur. In addition, extravasations of protein and fluids into the extra-vascular space will lead to the formation of edema and creation of new effusions.

Medical management of CLS begins with carefully monitoring of the patient's fluids and organ perfusion status. This is achieved by frequent determination of blood pressure and pulse, and by monitoring organ functions, which includes assessment of mental status and urine output. Occurrence of hypovolemia will be monitored. Flexibility in fluid and pressor management is essential for maintaining organ perfusion and blood pressure. Therefore, extreme caution should be used in treating

patients with fixed requirements for large volumes of fluid. Administration of IV fluids, either colloid or crystalloid is recommended for treatment of hypovolemia. Correction of hypovolemia may require large volumes of IV fluids but caution is required because unrestrained fluid administration may exacerbate problems associated with edema formation or effusions. With extra-vascular fluid accumulation, edema is common and ascites, pleural or pericardial effusions may develop. Management of these events depends on a careful balancing of the effects of fluid shifts so that neither the consequences of hypovolemia (e.g., impaired organ perfusion) nor the consequences of fluid accumulations (e.g., pulmonary edema) exceed the patient's tolerance. Pulmonary edema, another manifestation of CLS, can lead to respiratory failure.

5.4.2. Hypomagnesemia

Hypomagnesemia is seen more frequently in subjects with cancer when compared to the general population. If observed in this study, hypomagnesemia should be treated as clinically appropriate according to local medical practice, and a serum chemistry panel and urine sample for fractional excretion of magnesium should be collected.

5.4.3. Coagulation

For patients on anticoagulant therapy, close monitoring of coagulation parameters is recommended during the study treatment phase.

5.4.4. Impaired kidney and liver functions

Kidney and liver functions may be impaired during ALT-803 treatment. Use of concomitant nephrotoxic or hepatotoxic medications may further increase toxicity to the kidney and liver.

5.4.5. Infection

Antibiotics can be administered to treat infections.

5.4.6. Fever and Chills

Acetaminophen, indomethacin and hydromorphone can be administered to reduce fever and chills. Recommended administration is described in Section 5.3.

5.4.7. Gastritis

H2 blockers can be administered to prevent gastritis.

5.4.8. Diarrhea, Nausea and Vomiting

Antidiarrheal can be used to combat diarrhea; antiemetics can be used to attenuate nausea and vomiting. Steroid-based anti-emetics are not allowed. Recommended administration for treatment of nausea and vomiting is described in Section 5.3.

5.4.9. Pruritus and Dermatitis

Hydroxyzine or diphenhydramine can be used to control the symptoms from pruritus. Topical creams and ointments should be applied as needed for skin manifestations. Preparations containing a steroid should be avoided.

5.4.10. Acidosis

Sodium bicarbonate (IV) can be used to manage plasma pH or acidosis, as part of a comprehensive medical plan administered by the treating physician.

5.4.11. Life Threatening Toxicities

Life-threatening toxicities, if they are attributed to inflammatory reactions of ALT-803 in the judgment of the investigators, may be ameliorated by the intravenous administration of dexamethasone or other steroid-based medications. However, the use of systemic steroid medications may result in loss of the therapeutic effects of the study drug. Patients receiving steroid medication may continue to receive the study treatment if, in the opinion of the Principal Investigator, there is potential clinical benefit and the patient is motivated to do so.

5.4.12. Other Supportive Care

Other supportive care deemed necessary by the Principal Investigator will be used to ensure patients' welfare. This will include any isolated deviations or modifications of the care plans outlined above. If it is the case that the same modifications are apparently needed more systematically, then a protocol modification will be considered in consultation with the Sponsor and co-investigators.

6. TREATMENT MODIFICATIONS

The treatment modifications below apply to all study treatment visits when nivolumab and/or ALT-803 are given. Where specified as delay or hold, the study treatment (nivolumab and/or ALT-803) will be delayed/held week to week.

Event	Grade/Severity	Study treatment modifications
Kidney dysfunction	eGFR< 45mL/min	Delay the scheduled study treatment one week each for up to 2 weeks until a recovery is sufficient to resume study treatment, otherwise, discontinue study treatment.
Febrile neutropenia		
Thrombocytopenia with bleeding or anemia		
ALC	≥ 50,000/μL	
WBC	≥ 60,000/μL	
Systolic Hypotension	< 90mm	Manage with bolus fluid administration of up to 1.5 L over 24 hours. If systolic hypotension (<90mm) of Grade <3 persists at time of next scheduled study treatment, an attempt should be made to correct this with bolus fluids administration of up to 1.5 L over 6 hours. If this corrects the hypotension, the study treatment can be given. If it does not correct, study treatment should be delayed one week each for up to 2 weeks until recovery is sufficient to resume study treatment, otherwise discontinue study treatment.
Allergic reactions/acute infusion reaction	Grade 2 with bronchospasm or any Grade 3-4	Discontinue study treatment.
	Grade 1 or Grade 2 (without bronchospasms)	Delay study treatment until symptoms subside.
Pneumonitis	2	Hold study treatment. Resume once improved to grade 1 or resolved.
	3-4	Permanently discontinue study treatment.
Colitis	2-3	Hold study treatment. Resume once improved to grade 1 or resolved.
	4	Permanently discontinue study treatment.
ALT or AST	> 3-8 X ULN	Hold study treatment. Resume once improved to grade 1 or resolved
	> 8 X ULN	Permanently discontinue study treatment.
Total Bilirubin	> 1.5-6 X ULN	Hold study treatment. Resume once improved to grade 1 or resolved
	> 6 X ULN	Permanently discontinue study treatment.

Treatment modifications for AEs not specified elsewhere in the protocol:

1. Any other grade 3 study treatment-related AEs: hold the study treatment, resume once improved to grade 1 or resolved.
2. Discontinue study treatment for any of the following:
    a. Event that meets DLT definition
    b. Any grade 3 study treatment-related AE that recurs

c. Inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks

7. DRUG INFORMATION

7.1. ALT-803 (IL-15N72D:IL-15RαSU/IGG1 FC COMPLEX)

7.1.1. Description

ALT-803 is a soluble complex consisting of two protein subunits of a human IL-15 variant associated with high affinity to a dimeric human IL-15 receptor α (IL-15Rα) sushi domain/human IgG1 Fc fusion protein (56)(64) The IL-15 variant is a 114 aa polypeptide comprising the mature human IL-15 cytokine sequence with an Asn to Asp substitution at position 72 of helix C (N72D) (64) The human IL-15Rα sushi domain/human IgG1 Fc fusion protein comprises the sushi domain of the human IL-15 receptor α subunit (IL- 15Rα) (aa 1-65 of the mature human IL-15Rα protein) linked with the human IgG1 CH2-CH3 region containing the Fc domain (232 amino acids). This IL-15RαSu/IgG1 Fc fusion protein has the advantages of dimer formation through disulfide bonding via IgG1 domains and ease of purification using standard Protein A affinity chromatography methods. Additionally, it has been demonstrated that intracellular complex formation with IL-15Rα prevents IL-15 degradation in the endoplasm reticulum and facilitates its secretion {{}} It was found that through a co-expression strategy in CHO cells, both the IL-15N72D and IL-15RαSu/IgG1 Fc proteins were produced at high levels and formed a soluble, stable complex (56) The biological activity of CHO-produced ALT-803 complex was equivalent to *in vitro* assembled IL-15N72D:IL-15RαSu/IgG1 Fc complexes in standard cell-based potency assays using IL-15-dependent cell lines. Thus, this method represents a better approach for generating active, fully characterized cGMP-grade IL-15:IL-15Rα complex than current strategies employing *in vitro* assembly of individually produced and, in some cases, refolded proteins. Aside from the N72D substitution, all of the protein sequences in ALT-803 are human.

7.1.2. Mode of Action

As detailed in the Background section, ALT-803 is an IL-15-based immunostimulatory protein complex that acts as a growth and activation factor for NK cells and effector and memory T cells. Based on the results of animal tumor models, ALT-803 stimulated cellular immune responses are expected to exhibit potent activity against human tumor cells. Due to the higher affinity single amino acid substituted IL-15 variant and the presentation of IL-15 with IL-15RαSu, ALT-803 is likely to be effective at low concentrations. Owning to its longer half-life as a result of fusion with Fc, ALT-803 is likely to be effective using a practical regimen of weekly or biweekly injections. It is likely that low concentrations of ALT-803 will increase the peripheral blood T cell and NK cell counts to predictable levels with a safety profile appropriate for outpatient use.

7.1.3. Toxicity

Toxicities observed in human trials with ALT-803 are described in Section 3.10.

7.1.4. Storage

ALT-803 will be stored at the site in a secured area at 2°C to 8°C with limited access and protection from excess light and heat.

7.1.5. Packaging

ALT-803 is provided in a 2mL vial containing 1.2mL or ALT-803 at a concentration of 1 mg/mL. Vials are packaged in cartons and shipped to the clinical site.

7.1.6. Labeling

ALT-803 will be labeled in accordance with 21 CFR 312.6 which includes the statement "Caution: New Drug – Limited by Federal (or United States) law to investigational use.

7.1.7. Treatment Administration

ALT-803 dose calculation will be based on patient's assigned dose level and ALT-803 dosing will be calculated using a weight obtained within 5 days prior to the first dose. . The dose will be re-calculated at the beginning of each subsequent cycle in the event of a 10% or greater weight change. Injection sites should be rotated per institutional guidelines and each injection site separated by at least 1 inch.

The calculated amount of ALT-803 will be drawn into a syringe for subcutaneous injection. The stock concentration is 1 mg/mL. Doses will be drawn directly into the syringe for injection. The dose will be divided into 2-3 subcutaneous injections as needed.

ALT-803 can be stored in a syringe for up to 24 hours at 4°C.

7.1.8. Safety Precautions

ALT-803 is a clear, odorless non-flammable aqueous solution. It does not meet OSHA criteria for hazardous materials. There are no special safety precautions for handling ALT-803.

7.1.9. Temperature Excursion

In the event of a temperature excursion, ALT-803 vialed drug remains stable at the elevated temperatures of 25°C and 37°C for up to 7 days and can withstand -20°C for up to three freeze thaw cycles.

7.1.10. Drug Destruction

Unused drug will be returned to Altor. A shipper and instructions will be provided at the end of the study. Used vials should be destroyed per institutional policy.

7.2. OPDIVO (NIVOLUMAB)

Nivolumab is commercially available. See package insert for comprehensive treatment information including, but not limited to, treatment preparation and administration as well as full pharmacologic and toxicity information.

7.2.1. Description

Nivolumab is a PD-1 blocking antibody indicated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor. Nivolumab was recently approved by the FDA for the treatment of patients with metastatic NSCLC with progression on or after platinum-based chemotherapy. Nivolumab blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2.

7.2.2. Toxicity

The most common (>10%) adverse events reported in patients treated with Nivolumab are: rash, pruritus, cough, upper respiratory tract infection and peripheral edema. Laboratory abnormalities reported in >10% of patients include increased AST, increased alkaline phosphatase, hyponatremia, increased ALT and hyperkalemia. Other important, but less common (<10%) adverse events reported are: ventricular arrhythmia, iridocyclitis, infusion-related reactions, increased amylase, increased lipase, dizziness, peripheral and sensory neuropathy, exfoliative dermatitis, erythema multiforme, vitiligo, and psoriasis.

There is also risk for immunogenicity. Less than 10% of patients tested positive for treatment-emergent anti-product antibodies by ECL assay. Neutralizing antibodies were detected in <1% of patients. There is no evidence of altered PK profile or toxicity profile with anti-product binding antibody development based on the PK and exposure-response analysis.

Based on its mechanism of action and animal data, nivolumab has been shown to cause fetal harm when administered to pregnant women. It is unknown if nivolumab is present in human breastmilk.

The effectiveness of nivolumab has not been established in pediatric population. There is not sufficient data available to determine if patients older than 65 respond differently than those under than 65.

8. CONCOMITANT MEDICATIONS

8.1. PERMITTED MEDICATIONS – USE WITH CAUTION

- CNS function may be affected. Interactions could occur following concomitant administration of psychotropic drugs, including narcotics, analgesics, antiemetics, sedatives and tranquilizers.

8.2. PROHIBITED MEDICATIONS

- Hypersensitivity reactions have been reported in patients receiving combination regimens containing sequential high dose IL-2 and antineoplastic agents, specifically, dacarbazine, cisplatin, tamoxifen and interferon-alfa. These reactions consisted of erythema, pruritus, and hypotension and occurred within hours of administration of chemotherapy. Concurrent chemotherapies and interferon-alfa with ALT-803 are prohibited.
- Myocardial injury, including myocardial infarction, myocarditis, ventricular hypokinesia, and severe rhabdomyolysis appear to be increased in patients receiving IL-2 and interferon-alfa concurrently. Exacerbation or the initial presentation of a number of autoimmune and inflammatory disorders have been observed following concurrent use of interferon-alfa and IL-2, including crescentic IgA glomerulonephritis, oculo-bulbar myasthenia gravis, inflammatory arthritis, thyroiditis, bullous pemphigoid, and Stevens-Johnson syndrome. Therefore, concurrent interferon-alfa treatment with ALT-803 is prohibited.
- Kidney and liver function may be impaired. Use of nephrotoxic, myelotoxic, cardiotoxic, or hepatoxic medications may increase toxicity to the kidney or liver. These medications should be avoided during treatment with ALT-803.
- Glucocorticoids have been shown to reduce cytokine-induced side effects including fever, renal insufficiency, hyperbilirubinemia, confusion and dyspnea, concomitant administration of these agents with ALT-803 may reduce the anti-tumor effectiveness of ALT-803. Therefore administration of glucocorticoids should be avoided during ALT-803 treatment.
- Beta-blockers and other antihypertensives may potentiate the hypotension seen with cytokine therapy. Therefore, administration of these medications should be avoided during the ALT-803 portion of the treatment period but may be used at the discretion of the treating physician when there is at least a 72 hours gap from hypotension to ALT-803 treatment.
- Delayed Adverse Reactions to Iodinated Contrast Media: 11-28% of patients treated with IL-2 containing regimens and were subsequently administered radiographic iodinated contrast media experienced acute, atypical adverse reactions. The onset of symptoms usually occurred within hours (most commonly 1 to 4 hours) following the administration of contrast media. These reactions include fever, chills, nausea, vomiting, pruritus, rash, diarrhea, hypotension, edema, and oliguria. Some clinicians have noted that these reactions resemble the immediate side effects caused by IL-2 administration, however the cause of contrast reactions after IL-2 therapy is unknown. Most events were reported to occur when contrast media was given within 4 weeks after the last dose of IL-2. These events were also reported to occur when contrast media was given several months after IL-2 treatment. Similar conditions may occur with ALT-803 treatment. Washout period between IV dye exposure and ALT-803 exposure is at the discretion of the investigator.

9. STUDY ASSESSMENTS

9.1. GENERAL CONSIDERATIONS

- Informed consent must be obtained prior to any study specific assessments.
- Radiology assessment will be completed within 28 days prior to registration
- All other screening and baseline evaluations will be performed within 14 days prior to registration.
- Vital signs (especially blood pressure), clinical and laboratory assessments will be completed before start of therapy.
- Patients will be monitored after ALT-803 treatment as described in this section. Patient monitoring following nivolumab treatment will follow institutional standard of care.
- Treatment may be administered +/- 1 day. Exceptions for an increased window may be made during holidays or due to inclement weather provided prior approval by the sponsor-investigator.
- During study treatment, safety labs can be drawn within 48 hours of scheduled dose.
- Body weight (actual) will be collected before dosing for each ALT-803 treatment day for all patients.
- Concomitant medications will be recorded until 30 days after the completion of the last dose of study treatment. Concomitant medication for medically significant adverse events, which are ongoing at the end of study treatment, should be followed until the adverse event is resolved or considered stable.

9.2. MEDICAL HISTORY AND PRIOR THERAPY

A complete medical history of all body systems and history of all prior therapies will be performed.

9.3. PREGNANCY TEST

Serum pregnancy test is for WOCBP.

9.4. PHYSICAL EXAM, PERFORMANCE STATUS

For screening a complete physical exam will be performed including head, eyes, ear, nose, and throat, neck, cardiovascular, chest/lungs, abdomen (including liver and spleen size), extremities, neurological, skin, and lymph nodes. For subsequent visits per the study calendar, a focused physical examination is sufficient, including abdomen (including liver and spleen size), lymph nodes, and any other system that may contribute to clinical disease assessments. An ECOG performance status will be assigned.

9.5. VITAL SIGNS, BODY WEIGHT & HEIGHT

During Phase I, after the first ALT-803 dose on study, patients will be observed for 6 hours post-dosing for immediate adverse events; during Phase II, after the first ALT-803 dose on study, patients will be observed for 2 hours post-dosing. See Section 5.3.1 for the vital assessment requirements during ALT-803 observation. Height will be measured at baseline; body weight will be measured at baseline and on each dosing day before dosing for all patients. Patient monitoring following nivolumab will follow established standard of care.

9.6. CARDIAC FUNCTION MONITORING

An EKG will be performed at screening. Cardiac function should be monitored at each visit by clinical examination and assessment of vital signs for hypotension, arrhythmia, angina and myocardial infarction. During the study, patients with signs or symptoms of chest pain, murmurs, gallops, irregular rhythm, or palpitations must be further assessed if clinically indicated, including the need for hospitalization.

9.7. CLINICAL BLOOD TESTS

- Blood counts: CBC with Differential.
- Blood chemistry: CMP (albumin, alkaline phosphatase, ALT, AST, BUN, calcium, carbon dioxide, creatinine, glucose, potassium, sodium, total bilirubin, total protein)
- T4, TSH, amylase and lipase
- Magnesium, phosphorus and LDH
- Hepatitis B and C serologies at screening in patients not previously tested or if testing was done greater than 2 years from registration.

9.8. STAGING RADIOLOGIC EVALUATION

- Contrast CT scans of the chest, abdomen and pelvis, MRI or PET-CT.
- Disease response and disease progression will be evaluated in this study using irRECIST criteria. Refer to Section 11 for details.
- The baseline disease assessment will be performed before the initiation of study treatment and response assessments will be performed as indicted in the study calendar.
- 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. If there is a change in modality, then the study site may be asked to explain the reason for the change in the eCRF. A change in modality may be considered a protocol deviation.
- Restaging may be completed up to +/-7 days. If a dose modification causes a delay in study drug administration, the schedule of radiographic assessment will remain every 42 days.

9.9. ADVERSE EVENTS

See Section 15 for details regarding recording and reporting of AEs.

9.10. STUDY CALENDAR

Test or Procedure	Screening/ Baseline[1]	ALT-803 + Nivolumab Cycles 1-4 (1 Cycle = 6 Weeks)							Subsequent Cycles (Nivolumab)			Q 42 days	End of Study[11]	Post Study
Week		W1		W2	W3	W4	W5	W6	W1	W3	W5			
Day (+/- 1 day)		D1	D4[14]	D1	D1	D1	D1	Rest	D1	D1	D1			
Assessments														
Medical history	X													
Serum Pregnancy Test for WOCBP	X													
Complete PE	X												X	
Focused PE		X				X			X					
ECOG PS	X	X				X			X				X	
Vital signs[2]	X	X		X	X	X	X						X	
Weight	X	X		X	X	X	X							
Concurrent Medications	X	X		X	X	X	X		X				X	
AE assessment[3]	X	X		X	X	X	X		X				X	
Side Effect Diary		X		X	X	X	X							
CBC with differential[4]	X	X	X[14]			X			X		X[13]		X	
CMP[4]	X	X							X				X	
Magnesium, Phosphorus, LDH[4]	X	X							X				X	
Free T4, TSH[4]	X	X							X				X	
Hepatitis B and C serologies[5]	X													
Radiologic Evaluation [9]	X							X				X[10]	X[10]	
EKG	X													
Disease and survival follow-up														X[8]
Correlative Research Studies														
Immunophenotyping of PBMC	**See section 9.11 for outline of correlative study collection.** X[7]													
Serum collection for PK, cytokine and immunogenicity														
Tumor Tissue Analysis (archival tissue)[6]														
Tumor Biopsy (biopsy cohort)[7]	X											X		
Drug Administration														
Nivolumab		X			X		X		X	X	X			

ALT-803		X		X	X	X	X						

1. Screening/baseline evaluations are performed ≤ 14 days prior to registration; baseline radiologic evaluation is performed ≤ 28 days prior to registration.
2. Vital signs will be evaluated at prior to treatment. On Cycle 1 Day 1, vitals will be monitored at the time of ALT-803 administration, 15, 30, 60 and 120 min then hourly post dose until discharge or at completion of dose monitoring. See Section 5.3.1 for more details.
3. Patients who have an on-going study treatment-related SAE upon study completion or at discontinuation of study will be contacted by the study team every week until the event is resolved or determined to be irreversible.
4. Safety labs can be drawn up to 48 hours prior to scheduled dose.
5. Hepatitis B and C screening is only required for those not previously tested or if testing was done more than 2 years prior to registration.
6. See Section 13.5 for instructions on tissue preparation for the tumor tissue analysis. Patients will not be excluded if they do not have tumor tissue available.
7. See Section 13.4 for patient selection for biopsy cohort. Up to 20 patients will be selected to have fresh tumor biopsies at baseline and at the *first* restaging at the end of the 6th week. The baseline biopsy can be done after informed consent and prior to start of treatment.
8. After end of study visit, PFS, OS, and duration of response of all treated patients will be assessed at least every 3 months (+/- 1 week) during years 1 and 2 and every 6 months (+/- 2 weeks) during year 3or until death, whichever occurs first (See Section 9.14). Information about tumor assessment & other therapies received after completion of study treatment will be collected if available.
9. Contrast CT scans of the chest, abdomen and pelvis, MRI or PET-CT. Radiologic evaluation may be done +/- 7 days. If a dose modification causes a delay in study drug administration, the schedule of this assessment will remain every 6 weeks (42 days). Patients with untreated brain metastases should have a brain MRI repeated every 6-8 weeks.
10. End of study visit will be conducted within 7 days of decision to permanently discontinue study. If a subject comes off study for radiographic disease progression, the end of study radiographic assessment is not required. However, if a subject is taken off study for other reasons, such as toxicity or patient decision, all attempts should be made to obtain a radiographic assessment at the end of study.

9.11. CORRELATIVE STUDY BLOOD DRAW COLLECTION TIMEPOINTS

See Section 13 and the 102323 Correlatives Manual for more details regarding sample collection and processing. If a correlative collection timepoint is missed, all attempts should be made to collect the samples form the subject prior to the next scheduled correlative collection. Missed correlative collections are considered protocol deviations. Delayed correlative collections will not be considered deviations, but the delay should be noted on the collection worksheet.

	Cycle 1					**Cycle 2**			**Cycle 3**		**Cycle 4**	**Cycle 5**	**EOS**
Week	**W1**		**W2**	**W3**	**W5**	**W1**		**W3**	**W1**	**W2**	**W1**	**W5**	
Day	D1	D4	D1	D1	D1	D1	D4	D1	D1	D1	D1	D1	
Correlative Study													
PBMC (30mL)	X	X	X	X		X	X		X			X	X
SERUM (5mL)	X[1]	X	X	X	X	X	X	X	X	X	X	X	X

1. During Phase Ib and Phase II, pre-dose serum blood draw will occur on day 1. **For Phase Ib only** – post ALT-803: 30 minute, 2 hours, 6 hours and 24 hours

9.12. CRITERIA FOR REMOVAL FROM STUDY TREATMENT

Patient may discontinue study treatment for any of the following events:

- All dose limiting toxicities
- Radiographic disease progression (see Section 9.14 for guidance regarding the continuation of treatment after disease progression)
- Clinical disease progression
- Inter-current illness or study treatment related toxicity that would, in the judgment of the investigator, affect assessments of clinical status to a significant degree or require discontinuation of study treatment
- Death
- Patient may withdraw from the study at any time for any reason.
- Treatment delay greater than 6 weeks for any toxicity
- Concomitant treatment with a prohibited medication
- Patient non-compliance

9.13. GUIDANCE FOR CONTINUATION OF STUDY TREATMENT BEYOND DISEASE PROGRESSION

Response will be assessed using immune-related RECIST Criteria definitions provided in Section 11.2. Because a minority of subjects treated with immunotherapy may derive clinical benefit despite initial evidence of PD the following guidance is provided for continuation of study treatment beyond disease progression (74).

Subjects receiving study treatment will be permitted to continue study treatment beyond initial immune-related RECIST Criteria defined PD, assessed by the investigator, as long as they meet the following criteria:

- Investigator-assessed clinical benefit, and do not have rapid disease progression.
- Tolerance of study drug
- Stable performance status
- Treatment beyond progression will not delay an imminent intervention to prevent serious complications of disease progression (eg, CNS metastases)
- Investigator notifies study subject of any reasonably foreseeable risks or discomforts, or other alternative treatment options. This notification should be documented by the study team in the subject's research chart.

The decision to continue treatment beyond initial investigator-assessed progression should be discussed with the sponsor-investigator and documented in the study records.

A radiographic assessment/ scan should be performed within 6 weeks of initial investigator-assessed progression to determine whether there has been a decrease in the tumor size or continued PD. The assessment of clinical benefit should be balanced by clinical judgment as to whether the subject is clinically deteriorating and unlikely to receive any benefit from continued treatment with study treatment.

If the investigator feels that the study subject continues to achieve clinical benefit by continuing treatment, the subject should remain on the trial and continue to receive monitoring according to the study calendar.

For the subjects who continue study therapy beyond progression, further progression is defined as an additional 10% increase in tumor burden from time of initial PD. This includes an increase in the sum of diameters of all target lesions and/ or the diameters of new measurable lesions compared to the time of initial PD. Study treatment should be discontinued permanently upon documentation of further progression.

New lesions are considered measureable at the time of initial progression if the longest diameter is at least 10 mm (except for pathological lymph nodes which must have a short axis of at least 15 mm). Any new lesion considered non-measureable at the time of initial progression may become measureable and therefore included in the tumor burden if the longest diameter increases to at least 10 mm (except for pathological lymph nodes which must have a short axis of at least 15 mm). In situations where the relative increase in total tumor burden by 10% is solely due to inclusion of new lesions which become measurable, these new lesions must demonstrate an absolute increase of at least 5 mm.
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 objective progression (ie, radiographic confirmation) even after discontinuation of treatment.

9.14. FOLLOW UP

Patients removed from study for unacceptable AE(s) will be followed for AE(s) for 24 weeks or until resolution or stabilization of the AE if that takes more than 24 weeks.

After end of study treatment, PFS, OS, and duration of response of all treated patients will be assessed at least every 3 months (+/- 1 week) during years 1 and 2 and every 6 months (+/- 2 weeks) during year 3. Information about disease response assessments and other therapies (chemotherapy, surgery, radiation therapy, other investigational treatment) received after discontinuation or completion of study treatment will be collected as available during the follow-up period. Tabulation of subsequent therapies is planned, but missing data or data obtained outside the scheduled follow-up window, will not be considered a protocol deviation.

Disease progression and survival follow-up will be conducted by the research staff to collect information including disease progression and survival for analysis of PFS and OS. Death related information includes date and cause. Data collection may occur via telephone contact with the patient, during subsequent clinical visits or from available patient medical records.

10. STATISTICAL CONSIDERATIONS

10.1. PHASE Ib

10.1.1. Description of dose finding design

A continual reassessment method (CRM) design will be used to identify the maximum-tolerated dose (MTD) (Cheung, 2011). The CRM has been shown to have better properties than the more commonly employed '3+3" design (75). Briefly, it assumes the probability of a DLT at dose *j* is equal to $\pi_j(\alpha) = p_j^{\exp(\alpha)}$ where the p_j values ($j = 1,2,3,4$ doses which correspond to 3 µg/kg, 6 µg/kg, 10 µg/kg and 15 µg/kg of ALT-803, respectively) are the "skeleton" for the model. It is an adaptive dose-finding approach where a model-based estimate of the dose is used to determine where the next cohort of patients should be treated based on the accumulated toxicity information from patients already treated on the trial. The prior distribution for the α parameter is normal with a mean of 0 and variance of 1.34 ($\alpha \sim N(0, 1.34)$).

Our CRM assumes a target DLT rate of 0.25, a maximum sample size of 21, cohorts of size 3, starts at dose level 1 (6 µg/kg). The R package `dfcrm` (76)was used to initially calibrate our skeleton based on our sample size, target DLT rate, the delta parameter, and cohort size. The delta parameter was selected to be 0.05 which can be interpreted to mean that an acceptable DLT rate is within +/- 0.05 of the target DLT rate. Therefore, our CRM is targeting a dose with a DLT rate of [0.20- 0.30]. Based on a logistic dose-toxicity assumption, we selected the skeleton to be $p = \{0.05, 0.13, 0.25, 0.38\}$ which is also our prior. After 21 patients have been treated, the dose which has an estimated DLT rate closest to 0.25 will be selected as the MTD.

Stopping rules: The design will stop when one of these criteria is met.

a) The sample size reaches 21
b) The recommended dose for the next cohort has already been given to 12 patients
c) The estimated DLT rate at dose -1 (3 µg/kg) is 0.40 or higher.

10.2. CRM OPERATING CHARACTERISTICS

Figure L shows four different true dose-response relationships considered to evaluate the behavior of the proposed CRM, labeled 1 through 4. The scenarios are chosen to reflect scenarios where there are both toxic doses and doses with DLT rates at or close to target DLT rate. The skeleton is shown in red and is similar to scenario 2. Simulations were performed where 3000 trials were simulated per scenario. The proportion of trials in which each dose level was chosen, the average number of patients treated at each dose level, and the average number of DLTs per dose level are shown in Tables 10.A-D. The results for the optimal dose per scenario are bolded. Overall, operating characteristics are quite good, especially considering a relatively small sample size (N=21). The optimal dose is chosen in the majority of the simulated trials for all scenarios. In scenarios 2 and 3, where one of the middle doses is optimal, the optimal dose is selected about 60% of the time with the lower and higher doses selected about 40% of the time. Selection of very toxic doses (DLT rate ≥

50%) or very nontoxic doses (DLT rate ≤10%) occurs very infrequently in all scenarios considered.

10.3. PHASE Ib ANALYSIS PLANS

The CRM design will guide us to a dose to select for expansion in the Phase II portion of the study. For each dose level, the DLT rate with a 95% confidence interval will be reported. Additionally, we will evaluate correlative outcomes.

Figure L: Four dose-toxicity association scenarios (1 through 4) considered for simulating operating characteristics of CRM design are shown in black. Skeleton is shown in red, and horizontal blue line indicates the target DLT rate (0.25). Scenario numbers (1-4) correspond to the scenarios described in the text and in Tables 10.A-D.

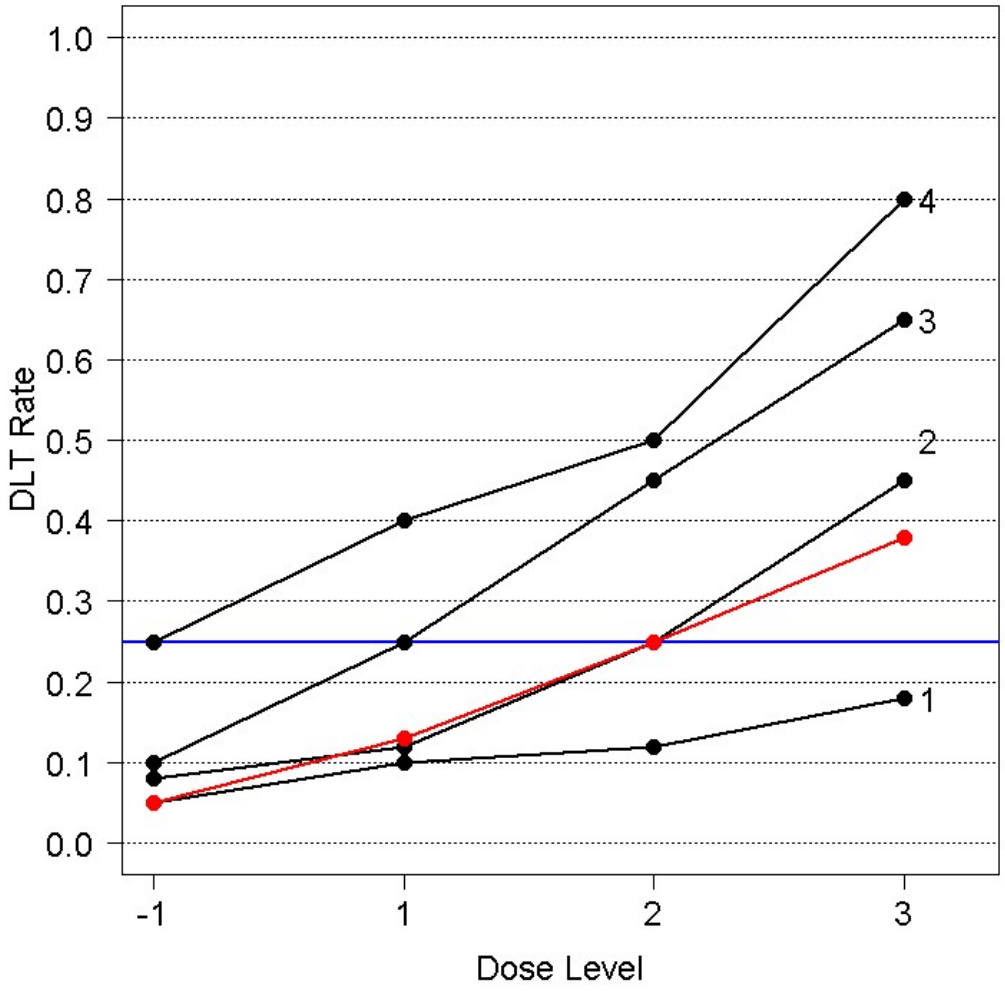


Table 10.A: Scenario 1

	Dose 1	Dose 2	Dose 3	Dose 4
True DLT rate	0.05	0.10	0.12	**0.18**
Probability dose selected	<0.01	0.06	0.19	**0.74**
Expected number of pts treated at dose	0.4	4.6	5.6	**8.4**
Expected number of toxicities observed at dose	<0.1	0.4	0.7	**1.5**
Expected Sample Size	19.1			
Expected Number of DLTs	2.6			
Expected percent of trials that stop early due to toxicity	<0.1%			
Expected percent of trials that stop early due to reaching max of 12 at one dose	56%			

Table 10.B: Scenario 2

	Dose 1	Dose 2	**Dose 3**	Dose 4
True DLT rate	0.08	0.12	**0.25**	0.45
Probability dose selected	0.02	0.21	**0.58**	0.18
Expected number of pts treated at dose	0.9	6.4	**8.3**	3.7
Expected number of toxicities observed at dose	0.1	0.8	**2.0**	1.7
Expected Sample Size	19.2			
Expected Number of DLTs	4.5			
Expected percent of trials that stop early due to toxicity	<1%			
Expected percent of trials that stop early due to reaching max of 12 at one dose	44%			

Table 10.C: Scenario 3

	Dose 1	**Dose 2**	Dose 3	Dose 4
True DLT rate	0.10	**0.25**	0.45	0.65
Probability dose selected	0.16	**0.64**	0.16	<0.01
Expected number of pts treated at dose	3.7	**9.5**	4.4	0.5
Expected number of toxicities observed at dose	0.4	**2.4**	2.0	0.3
Expected Sample Size	18.1			
Expected Number of DLTs	5.1			
Expected percent of trials that stop early due to toxicity	3%			
Expected percent of trials that stop early due to reaching max of 12 at one dose	54%			

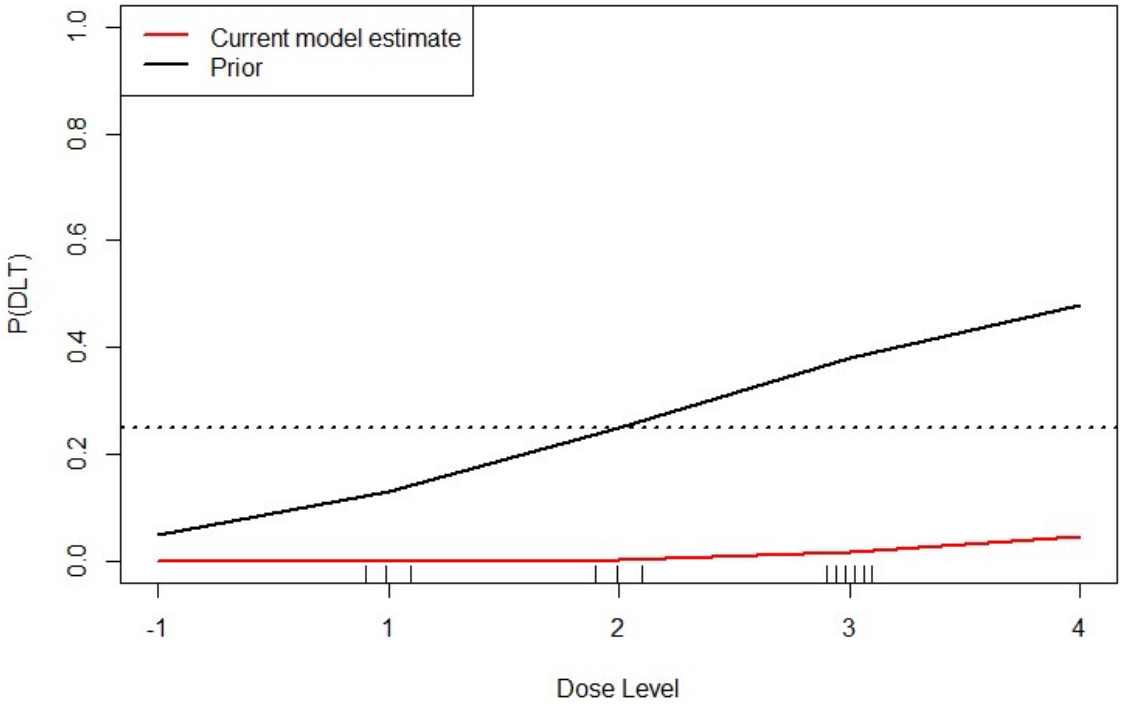
Table 10.D: Scenario 4

	Dose 1	Dose 2	Dose 3	Dose 4
True DLT rate	**0.25**	0.40	0.50	0.80
Probability dose selected	**0.48**	0.26	0.03	<0.01
Expected number of pts treated at dose	**6.8**	7.1	1.7	0.1
Expected number of toxicities observed at dose	**1.7**	2.9	1.0	0.1
Expected Sample Size	15.7			
Expected Number of DLTs	5.5			
Expected percent of trials that stop early due to toxicity	23%			
Expected percent of trials that stop early due to reaching max of 12 at one dose	46%			

10.3.1. Addition of Dose Level 4

Given the lack of toxicity at any of the current dose levels, a higher dose level (dose level 4) of 20 µg/kg has been added with Protocol Amendment 6 and assumes that no patient in dose level 3 has a DLT. The operating characteristics of the design have been explored given the trial design (including the prior, predefined max sample size of 21 treated patients, and the data observed thus far). To be able to expand the design to include a 5th dose level, an additional value for dose level 4 must be added to the skeleton and the prior. The previous skeleton and the prior (for doses -1 through 3) was $p = \{0.05, 0.13, 0.25, 0.38\}$. We have updated it to be $p = \{0.05, 0.13, 0.25, 0.38, 0.50\}$ for doses -1 through 4, which essentially provides a conservative estimate that we expect a 50% DLT rate at dose level 4. Based on the updated prior, the observed data, and the proposed CRM approach, the next cohort of 3 patients would be treated at the new dose level (20 µg/kg). See Figure M for current model estimation and prior.

Figure M: Graphical display of current observed data (shown via tick marks), the current dose-toxicity relationship based on the CRM design (red line), and the assumed prior for the dose-toxicity relationship (black line).

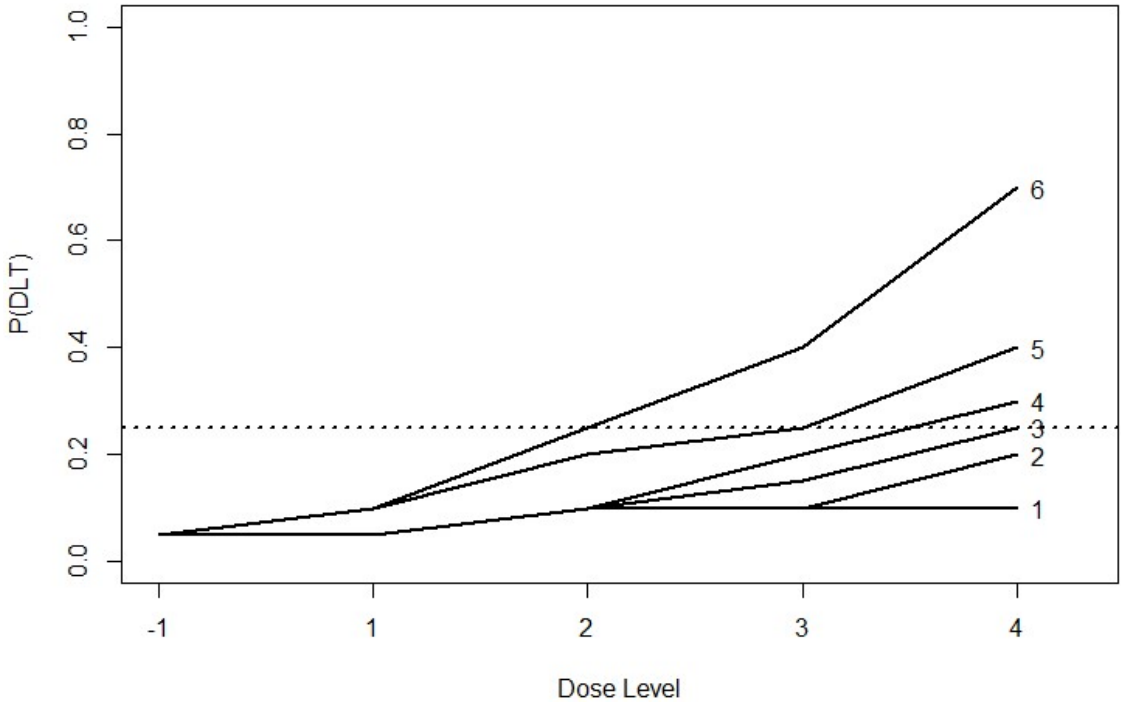


Operating characteristics show how the trial will proceed given that an additional 9 patients will be treated and the probability of choosing dose level 2, 3, or 4 as the MTD. We have considered 6 possible true toxicity scenarios ranging from very low toxicity across all dose levels (scenario 1) to one with toxicity above the target level for dose levels 3 and 4 (>=40% at dose levels 3 and 4). These scenarios are shown in Table 10.E and Figure N.

Table 10.E: Toxicity scenarios used for simulating data from the last 9 patients to be enrolled and treated. Numbers in the table represent the probability of a toxicity at each dose level within each of 6 scenarios. E.g. under scenario 4, the probability of a DLT at dose level 2 is 0.10.

	Dose Levels				
Scenario	-1	1	2	3	4
1	0.05	0.05	0.10	0.10	0.10
2	0.05	0.05	0.10	0.10	0.20
3	0.05	0.05	0.10	0.15	0.25
4	0.05	0.05	0.10	0.20	0.30
5	0.05	0.10	0.20	0.25	0.40
6	0.05	0.10	0.25	0.40	0.70

Figure N: Toxicity scenarios used for simulating data from the last 9 patients to be enrolled and treated.



Using the data from the first 12 patients treated, and simulating data from the CRM design for the remaining 9 patients to be treated, we demonstrate the operating characteristics of the trial in Table 3. If the trial continues and all doses have low/acceptable toxicity (consistent with scenarios 1-3), there is a 96% or greater chance that dose level 4 will be the selected MTD. If the toxicity curve is consistent with scenario 4, there is a 90% chance the dose level 4 would be selected as the MTD (with a true P(DLT) of 0.30) vs. 10% for dose level 3 (with a true P(DLT) of 0.20). Scenario 5 demonstrates that dose level 4 is still likely to be selected with a probability of 0.75 is the P(DLT) is 0.40. However, when the DLT rate is 0.70 (scenario 6) at dose level 4, the probability of selecting it is only 0.17. Also shown in Table 3 is the expected distribution of the 21 patients across all dose levels. For scenarios 1, 2, 3 and 4, it is expected that all nine patients will be treated at dose 4. For scenario 5, the average number of patients treated at dose level 4 is 8.3. For scenario 6, it is expected that 6 of the 9 patients will be treated at dose level 4, and 3 of the nine patients will be treated at dose level 3.

Table 10.F: Operating characteristics for the CRM design based on the proposed amendment to add dose level 4 (15 μg/kg) and maintain maximum sample size of patients treated of N=21. Toxicity scenarios referred to in the table are shown in Figure N.

	Average number of patients at each dose level (including the 12 patients already enrolled)					Probability that dose is chosen as MTD		
Toxicity Scenario	Dose level -1	Dose level 1	Dose level 2	Dose level 3	Dose level 4	P(MTD=2)	P(MTD=3)	P(MTD=4)
1	0	3	3	6	9.0	--	--	**100%**
2	0	3	3	6.1	8.9	--	1%	**99%**
3	0	3	3	6.1	8.9	--	4%	**96%**
4	0	3	3	6.2	8.8	--	**10%**	**90%**
5	0	3	3	6.7	8.3	1%	**25%**	74%
6	0	3	3.1	8.9	6.0	**13%**	70%	17%

Note: If the first three patients (pts 13, 14, and 15) treated at dose level 4 have no DLTs, then the next three patients (pts 16, 17 and 18) will be assigned (as per the model) to dose level 4, for a total of 6 patients at dose level 4. Even if all three patients in the 2nd cohort of patients (pts 16, 17, 18) at dose level 4, have DLTs (for an empirical DLT rate of 50% = 3/6), the model will assign patients 19, 20 and 21 to dose level 4. Thus, if patients 13, 14, and 15 have no DLTs at dose level 4, the DLT status of patients 16, 17 and 18 will not affect the dose assignment for patients 19, 20, and 21. Thus, if the first three patients at dose level 4 have no DLTs, we will plan to enroll the last 6 patients as one cohort (with no pause in enrollment for patients 16 through 21.

10.4. PHASE II

Based on the results from Phase Ib, a recommended phase II dose will be selected for the Phase II part of the study.

10.4.1. Study design overview

In Phase II, we will have two cohorts of patients: nivolumab, pembrolizumab or atezolizumab naïve (cohort A) and nivolumab, pembrolizumab or atezolizumab refractory/resistant (cohort B). In each cohort, a predictive probability phase II design will be used with objective response rate (ORR) as the outcome (1). These designs will allow early stopping for futility beginning with the 9th patient and then after every 3rd patient treated on the trial. In this approach, the ORR is assumed to follow a binomial distribution, and we assume the prior on the ORR follows a beta distribution, leading to a beta-binomial posterior distribution for the number of responses. The predictive probability (PP) is the probability of concluding a positive result at the end of the trial based on the information that has accumulated from the patients treated thus far. If PP is high (e.g. PP > 0.90), it suggests there is strong evidence that the null hypothesis will be rejected at the trial's end. If PP is low (e.g. PP < 0.10), it suggests that it is unlikely that the trial will reject the null hypothesis by the end of the trial and the study should be stopped for futility.

10.4.2. Phase II Design, Nivolumab, Pembrolizumab or Atezolizumab Naïve Patients (Cohort A)

ORR is the primary outcome and we define a null ORR of 0.15 and an alternative ORR of 0.35. A maximum of 40 patients (which includes any patients in the phase Ib trial who fit the eligibility criteria and are treated at the phase II dose level) will be included in the phase II portion of the study. The proposed trial has power of 89% and an alpha level of 0.056. The trial would stop early for futility after the 9th, 12th, 15th,…, 39th patient if the predictive probability (PP) of rejecting the null hypothesis at the end of the study is less than 5%. At the end of the study, the null hypothesis would be rejected if the posterior probability is greater than 0.90 and we would accept the alternative hypothesis. A weak prior was used for the ORR (a=0.2 and b=0.80), which is equivalent to information on one patient with a mean at an ORR of 0.20.

Stopping criteria: Stop the study if $PP_j < 0.05$ where $j = 9, 12, 15, ..., 39$ and PP_j is the predictive probability based on the accumulated ORR information from the 1^{st} through the j^{th} patient.

Decision rule at N=40 patients: Reject the null hypothesis if the posterior probability that the response rate is greater than 0.15 is at least 0.90. This corresponds to rejecting the null if there are at least 10 responses in 40 patients (an observed response rate of 0.25).

Operating characteristics of this design are shown in Table 10.E. Stopping boundaries of this design and probabilities of stopping at each evaluation of the data are shown in Table 10.H. As stated above, this design has an alpha of 0.056 and a power of 89%.

Table 10.G: Probability of early termination and expected sample size for the predictive probability design for Cohort A.

	Operating Characteristics	
	Null is True: ORR = 0.15	Alternative is True: ORR = 0.35
Probability of Early Termination	0.91	0.09
Expected Sample Size	20.8	38.2

Table 10.H: Operating characteristics of predictive probability design for Cohort A under the null hypothesis of an ORR of 0.15. H0: p =0.15 vs. H1: p = 0.35. Type I error of 6%, power of 89%. Maximum N=40. Predictive probability is calculated starting with the 9th patient and then after every third patient enrolled. A weak beta prior (Beta(0.2, 0.8) is used for the ORR.

		Operating Characteristics			
		Null is True: ORR = 0.15		Alternative is True: ORR = 0.35	
Patient number	Stop the study if the number of responses is less than or equal to:	Probability of Stopping	Probability of Continuing	Probability of Stopping	Probability of Continuing
9	0	0.23	0.77	0.02	0.98
12	1	0.23	0.54	0.03	0.95
15*	1	0	0.54	0	0.95

18	2	0.11	0.44	0.008	0.94
21*	2	0	0.44	0	0.94
24	3	0.07	0.37	0.003	0.94
27	4	0.08	0.29	0.005	0.94
30	5	0.07	0.23	0.006	0.93
33	6	0.06	0.17	0.007	0.92
36	7	0.04	0.12	0.007	0.92
39	8	0.03	0.09	0.007	0.91

*Data evaluations at 15 and 21 are not meaningful: the study would have stopped at an earlier time point given the boundary and so the probability of early stopping at these evaluation times is 0.

10.4.3. Description Phase II Design, Nivolumab, Pembrolizumab or Atezolizumab Refractory/Resistant Patients (Cohort B)

ORR is the primary outcome and we define a null ORR of 0.04 and an alternative ORR of 0.20. A maximum of 30 patients (which includes any patients in the phase Ib trial who fit the eligibility criteria and are treated at the phase II dose level) will be included in the phase II portion of the study. The proposed trial has power of 83% and an alpha level of 0.07. The trial would stop early for futility after the 9th or the 21st patient if the predictive probability (PP) is less than 10%. Note that there are only two stopping boundaries: (1) if there are 0 responses in the first 9 patients, the study will stop; (2) if there is only 1 response in the first 21 patients, the study will stop. At the end of the study, the null hypothesis would be rejected if the predictive probability is greater than 0.85 and we would accept the alternative hypothesis. A weak prior was used for the ORR (a=0.1 and b=0.90), which is equivalent to information on one patient with a mean at an ORR of 0.10.

Stopping criteria*:* Stop the study if $PP_j < 0.10$ where $j = 9, 21$ and PP_j is the predictive probability based on the accumulated ORR information from the 1^{st} through the j^{th} patient.

Decision rule at N=30 patients (if the study reaches N=30)*:* Reject the null hypothesis if the posterior probability that the response rate is greater than 0.04 is at least 0.91. This corresponds to rejecting the null if there are at least 4 responses in 30 patients (an observed response rate of 0.13).

Operating characteristics of this design are shown in Table 10.E. Stopping boundaries of this design and probabilities of stopping at each evaluation of the data are shown in Table 10.I. As stated above, this design has an alpha of 0.07 and a power of 83%.

Table 10.I: Probability of early termination and expected sample size for the predictive probability design for Cohort B.

	Operating Characteristics	
	Null is True: ORR = 0.04	Alternative is True: ORR = 0.20
Probability of Early Termination	0.85	0.16
Expected Sample Size	14.0	27.0

Table 10.J: Operating characteristics of predictive probability design for Cohort H0: p =0.04 vs. H1: p = 0.20. Type I error of 7%, power of 83%. Maximum N=30. Due to the discrete sample space and low expected response rate under the null, there are only two times of evaluation (N=9 and N=21). A weak beta prior (Beta(0.1, 0.9) is used for the ORR.

		Operating Characteristics			
		Null is True: ORR = 0.04		Alternative is True: ORR = 0.20	
Patient number	Stop the study if the number of responses is less than or equal to:	Probability of Stopping	Probability of Continuing	Probability of Stopping	Probability of Continuing
9	0	0.69	0.31	0.13	0.87
21	1	0.16	0.15	0.02	0.85

10.4.4. Early Stopping Boundaries for Safety

In either Phase II cohort, we would want to stop the study early if it were found that the combination (i.e. the MTD from phase I) is overly toxic. Although our phase I trial has been demonstrated to have good properties for identifying the correct MTD, there is still the chance that the MTD will be too toxic.

The following rules will be followed to stop the trial early due to toxicity based on the definition of DLT defined in section 5.2. This approach is based on a sequential likelihood approach (77), where we posit that an acceptable DLT rate is 15% and an unacceptable rate is 30%. If the likelihood ratio is 8 or greater in favor of the 30% rate, then the study will stop due to toxicity.

Operating characteristics in Cohort A (maximum N=40): If the true rate of DLT is 0.30, then the trial has a 72% chance of stopping early for toxicity (assuming the study does not stop for futility). If the true rate of DLT is 0.15, then the trial has a 7% chance of early stopping due to excessive toxicity

Operating characteristics in Cohort B (maximum N=30): If the true rate of DLT is 0.30, then the trial has a 62% chance of stopping early for toxicity (assuming the study does not stop for futility). If the true rate of DLT is 0.15, then the trial has a 6% chance of early stopping due to excessive toxicity.

This approach leads to the stopping boundaries in Table 10G.

Table 10K. Toxicity stopping boundaries. For example, if DLTs are observed in 4 of the first 7 patients, then the trial would stop for toxicity.

Number of Toxicities Observed	Accrued Sample Size	Observed Toxicity Rate	Likelihood Ratio
3	3	1.00	8.0
4	≤ 7	0.57	8.9
5	≤ 12	0.42	8.2
6	≤ 16	0.38	9.2

7	≤ 21	0.33	8.4
8	≤ 25	0.32	9.4
9	≤ 30	0.30	8.7
10	≤ 34	0.29	9.7
11	≤ 39	0.28	8.9
12	≤ 44	0.27	8.2
13	≤ 48	0.27	9.2
14	≤ 50	0.28	15.1

10.4.5. Analysis Plan for Phase II:

Based on the results from the predictive probability designs, we will have an estimated ORR and a posterior distribution for the ORR for each cohort. Toxicity outcomes will be tabulated by system organ class and grade.

11. MEASURES OF EFFECT

11.1. DEFINITIONS

Evaluable for Toxicity. All patients will be evaluable for toxicity from the time of their first treatment with drugs on study.

Evaluable for objective response. Only those patients who have measurable disease present at baseline, have received at least one 6-week cycle of ALT-803 plus nivolumab 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 prior to the end of cycle 1 will also be considered evaluable.)

11.2. RESPONSE GUIDELINES (RECIST 1.1)

Response will be assessed using immune-related RECIST Criteria definitions provided in Section 11.3. Because these definitions rely heavily on RECIST 1.1, these guidelines are provided in Section 11.2 for reference to facilitate categorization as defined in Section 11.3. For guidance on continuation of study therapy beyond progression refer to Section 9.13.

11.2.1. Disease parameters

Measurable Tumor Lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

- 10 mm by CT scan (CT scan slice thickness no greater than 5 mm).
- 10 mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
- 20 mm by chest X-ray.

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

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

Bone lesions: Bone scan, PET scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions.

Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross sectional imaging techniques such as CT or MRI can be

considered as measurable lesions if the soft tissue component meets the definition of measurability described above.

Blastic bone lesions are non-measurable.

Cystic lesions: 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 non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Brain Lesions: Brain lesions detected on brain scans can be considered as both target or non-target lesions.

Lesions with prior local treatment: Tumor lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion.

11.2.2. Documentation of Target and Non-Target Lesions

Target lesion selection. 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 selection: 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.

New Measurable Lesions: Must be a minimum of 10mm in the longest diameter for non-nodal lesions and a minimum 15mm in short axis for lymph nodes. Smaller lesions contribute to the non-target or new non-measurable tumor burden, but do not get measured.

11.3. IMMUNE RELATED RECIST CRITERIA (78):

11.3.1. Baseline: Measureable Lesion Definition and Target Lesion Selection

Follow the definitions from RECIST 1.1

11.3.2. Baseline: Non-Measurable Lesion Definitions

Follow the definitions from RECIST 1.1

11.3.3. Baseline: Target and Non-Target Lymph Node Lesion Definitions

Follow the definitions from RECIST 1.1

11.3.4. Baseline: Non-Target Lesion Selection

All lesions or sites of disease not recorded as target lesions should be recorded as non-target lesions at baseline. There is no limit to the number of non-target lesions that can be recorded at baseline.

11.3.5. Baseline: Bone Lesions

Follow the definitions from RECIST 1.1

11.3.6. Baseline: Brain Lesions

Brain lesions detected on brain scans can be considered as both target or non-target lesions.

11.3.7. Baseline: Cystic and Necrotic Lesions as Target Lesions

Lesions that are partially cystic or necrotic can be selected as target lesions. The longest diameter of such a lesion will be added to the Total Measured Tumor Burden (TMTB) of all target lesions at baseline. If other lesions with a non-liquid/non-necrotic component are present, those should be preferred.

11.3.8. Baseline: Lesions with Prior Local Treatment

During target lesion selection the radiologist will consider information on the anatomical sites of previous intervention (e.g. previous irradiation, RF-ablation, TACE, surgery, etc.). Lesions undergoing prior intervention will not be selected as target lesions unless there has been a demonstration of progress in the lesion.

11.3.9. Follow Up: Recording of Target and New Measurable Lesion Measurements

The longest diameters of non-nodal target and new non-nodal measurable lesions, and short axes of nodal target and new nodal measurable lesions will be recorded. Together they determine the TMTB at follow-up.

11.3.10. Follow Up: Definition of Measurable New Lesions

In order to be selected as new measurable lesions ($\leq$ 2 lesions per organ, $\leq$ 5 lesions total, per time point), new lesions must meet criteria as defined for baseline target lesion selection and meet the same minimum size requirements of 10 mm in long diameter and minimum 15 mm in short axis for new measurable lymph nodes. New measurable lesions shall be prioritized according to size, and the largest lesions shall be selected as new measured lesions.

11.3.11. Follow Up: Non-Target Lesion Assessment

The RECIST 1.1 definitions for the assessment of non-target lesions apply.

11.3.12. Follow Up: New Non-Measurable Lesions Definitions and Assessment

All new lesions not selected as new measurable lesions are considered new non-measurable lesions and are followed qualitatively. Only a massive and unequivocal progression of new non-measurable lesions leads to an overall assessment of irPD for the time point. Persisting new non- measurable lesions prevent irCR.

11.3.13. irRECIST Criteria Overall Tumor Assessments

Complete Response (irCR) - Complete disappearance of all measurable and non-measurable lesions. Lymph nodes must decrease to < 10 mm in short axis. Confirmation of response is not mandatory.

Partial Response (irPR) - Decrease of ≥ 30% in TMTB relative to baseline, non-target lesions are irNN[a], and no unequivocal progression of new non-measurable lesions.

Stable Disease (irSD) - Failure to meet criteria for irCR or irPR in the absence of irPD.

Progressive Disease (irPD) - Minimum 20% increase and minimum 5 mm absolute increase in TMTB compared to nadir, or irPD for non-target or new non-measurable lesions.

a. irNN – no target disease identified at baseline and at follow-up the patient fails to meet criteria for irCR or irPD

11.4. METHODS FOR EVALUATION OF MEASURABLE DISEASE

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.

CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, 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 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.

11.5. 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.

11.6. DURATION OF RESPONSE

Duration of Overall Response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (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: Stable disease 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.

11.7. PROGRESSION-FREE SURVIVAL

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

11.8. OBJECTIVE RESPONSE RATE

ORR is defined as the percentage of patients who achieve complete or partial response as defined by immune related RECIST criteria.

12. DEFINITION OF ENDPOINTS

12.1. PRIMARY ENDPOINT

1) For the phase Ib portion of the study the primary endpoint is dose-limiting toxicity as defined in Section 5.2.

2) For the phase II portion of the study the primary endpoint is objective response using immune related RECIST criteria as defined in Section 11.

12.2. SECONDARY ENDPOINTS

1) Secondary endpoints for both phases of the trial ALT-803 Cmax, AUC, immunogenicity, plasma cytokine concentration, and lymphocyte subpopulation characterization.

2) To define the progression-free survival, overall survival, and duration of response of all treated patients.

12.3. EXPLORATORY ENDPOINTS

Blood will be collected pre-therapy, during therapy and post- therapy for corollary studies and PK studies as described in Section 13.

12.4. SAFETY ENDPOINTS

All patients will be evaluable for toxicity from the time of their first treatment with drugs on study.

All patients will be monitored and evaluated for clinical toxicities during the treatment period and queried at each response assessment visit for AEs. All adverse events will be graded by using the NCI CTCAE v.4.0 and logged in the patient CRF. The study center should report all SAEs and all events that trigger patient's study treatment discontinuation to the Sponsor-Investigator via phone, fax or email (or a combination) no more than 24 hours after learning of the event. The Sponsor-Investigator will use the information to manage and coordinate the dose escalation and patient enrollment. The Sponsor-Investigator will then inform Altor Bioscience and all of the participating clinical sites of the current dose level and the number of patients to be enrolled at that level, or of any patient enrollment suspension via phone, fax or email within 24 hours of learning of the event. The study center should report all other adverse events to the Sponsor-Investigator following the guidelines defined in the study protocol. All study treatment related AEs that are both serious and unexpected will be reported to the FDA in an expedited manner in accordance with 21 CFR §312.32

13. BIOMARKER AND CORRELATIVE STUDIES

Please see the MUSC102323 Correlatives Studies Manual for additional collection, processing and shipping information. Note that for all of the correlative studies described in this section, all attempts will be made to collect research samples. If a correlative collection timepoint is missed, all attempts should be made to collect the samples form the subject prior to the next scheduled correlative collection. Missed correlative collections are considered protocol deviations. Delayed correlative collections will not be considered deviations, but the delay should be noted on the collection worksheet.

Samples will be linked to study subjects by the unique study ID provided at baseline. The facilities running the correlative study analysis will not have access to the subject's personal health information.

Each participating center will be responsible for the initial processing and storage of the samples for the correlative studies described in sections 13.1, 13.2 and 13.3.

Samples collected at MUSC will be stored by the CCT. Samples collected at other participating centers will be batch shipped to MUSC at designated intervals for storage until analysis is conducted. After shipment, the samples will be stored at the CCT and samples designated for analysis by Altor will be shipped to Altor upon request. The laboratory of Mark Rubinstein will also have access to samples, including processed PBMCs, serum, and plasma. The Rubinstein laboratory will use these samples for standard immune- and chemical- analysis including flow cytometry, ELISAs, and multiplex bead array technologies. In some cases, the Rubinstein laboratory will freeze these samples for subsequent analysis. For each collection, up to 20 million PBMCs, up to 3mls of serum, and/or up to 15mls of plasma, will be provided to the Rubinstein laboratory by the CCT. The remaining samples will be used by the CCT.

13.1. IMMUNOPHENOTYPING OF PBMC

The major focus will be on changes in WBC and lymphocyte counts, NK- and T-cell counts, and immune function in response to ALT-803. WBC, lymphocyte, NK-, and T-cell counts will be determined and reported as the absolute increase in each cell subset and variance in change across each dose level (mean, median, and standard error [SE] or standard deviation [SD]) for each dose level.

The primary goal of the correlative analysis is to evaluate the change in frequency of T and NK cells resulting from ALT-803 treatment. All measurements of effects on patient T-cell, NK-cell, and immune responses will be performed using peripheral blood and PBMC.

WBC counts and ALC, as part of the safety CBC with differential, will be evaluated as specified in the Study Calendar. Assays for and timing of NK- and T-cell counts are listed in section 13.5.

These samples will be sent to the MUSC central laboratory for subsequent analysis of T-cell and NK-cell number and immune response. The expansion of $CD4^+$ and $CD8^+$ T cells ($CD3^+CD4^+CD56^-$ and $CD3^+CD8^+CD56^-$) and NK cells ($CD3^-CD56^+$) as measured by flow cytometry will serve as key endpoints for assessing ALT-803+ nivolumab induced immune

responses. Although important for the assessment of treatment effect, in this trial these data will not be used for real-time clinical decision-making.

Levels of blood B cells ($CD19^+$), monocytes ($CD14^+$), NKT cells ($CD56^+CD3^+$), dendritic cells ($HLA\text{-}DR^+$ $CD123^+$ or $CD11c^+$), and CD122 (IL-15Rβ)-expressing cells will also be determined. An additional phenotyping panel will quantify the proportion of memory and naïve T cell subsets using antibodies to CD44, CD45RA, CCR7, and CD28, of Tregs using antibodies to CD127, CD4 and CD25, and the activation state/functionality of NK and T cells using antibodies to PD-1, ICOS, NKG2D, CD16 and MHC Class II (HLA-DR). In addition proliferation of immune cells will be assessed using a panel of antibodies recognizing Ki-67, CD3, CD56, CD4, CD8, and CD19. The absolute and percent change in each parameter and variance in change over time for each patient (mean, median, and SE/SD) will be evaluated.

For the phenotypic analyses, the absolute number and percentage of cells positive for the marker and/or mean fluorescence intensity (MFI) at time points after ALT-803 + nivolumab administration will be compared to baseline and the change will be calculated as #after/#baseline, %after/%baseline or MFI after/MFI baseline.

In other exploratory studies, flow cytometry may also be used to analyze the other phenotypic and functional properties of defined populations of peripheral leukocytes, for example, by challenging cells with mitogens or toll-like receptor agonists and measuring degranulation or cytokine responses (e.g., IFN-γ and IL-10) from T and NK cells. Samples will be sent to Adaptive Bioscience for genetic analysis for the presence of T cell-receptor.

13.2. SERUM SAMPLE ANALYSIS

The collected serum samples will be divided into four aliquots, as outlined in the correlative studies manual, and will be frozen and stored at the participating center. At designated intervals, the samples will be shipped to and stored at MUSC. Samples will be collected at the time points outlined in section 13.5. Residual samples may be used by MUSC for research studies of other biomarkers.

13.2.1. Pharmacokinetics (PK)

PK analysis will be performed at Altor Bioscience Corporation. PK analysis will only be conducted in subjects enrolled to the Phase Ib portion of the study. The PK profile of ALT-803 will be assessed using blood collected at the time points outlined in section 13.5 for the **first** ALT-803 dose.

Upon request, MUSC will ship one aliquot to Altor Bioscience upon request for evaluation using validated ELISA methods for PK analyses. In the PK assays, an IL-15-specific antibody is used as a capture antibody. Samples containing ALT-803 are applied and allowed to bind. After the appropriate wash conditions, labeled human IL-15 or IgG1-specific antibodies are used for detection with colorimetric ELISA substrates. For analysis of clinical samples, purified ALT-803 reference standards and control human serum containing high and low levels of added ALT-803 are included, and the level of ALT-803 in patient samples is determined based on the ALT-803 standard curve. Residual samples may be used by MUSC for research studies of other biomarkers.

13.2.2. Serum cytokine Assays

Changes in the plasma cytokine concentration of pro-inflammatory and immunosuppressive cytokines (e.g., IL-1B, IFN, IL-6, IL-10, TNF IL-8, IL-17 and IL-12) may correlate with the administration of ALT-803. The blood collected at each time point outlined in section 13.3 will be used by MUSC for multiplex cytokine ELISAs.

13.2.3. Immunogenicity

Altor Bioscience will conduct evaluation using validated ELISA methods. In these tests, human anti-ALT-803 antibodies are detected in patient serum samples using a direct sandwich ELISA method with plates coated with ALT-803. After the appropriate wash conditions, labeled ALT-803 is used for detection with colorimetric ELISA substrates. For analysis of clinical samples, anti-IL-15 antibody serve as reference standard and serum from ALT-803 immunized mice serve as a positive control. The level of anti-ALT-803 antibody in patient samples is determined based on the anti-IL-15 antibody standard curve. Similar processing and testing will be developed for anti-nivolumab antibody testing. Residual samples may be used by MUSC for research studies of other biomarkers.

13.3. OUTLINE OF CORRELATIVE STUDIES

Correlative Study Analysis	Timing of Collection	Amount of research blood collected
Immunophenotyping of PBMC (section 13.1). All collections are predose.		
Peripheral blood WBC count Peripheral blood ALC	Same as CBC with differential per study calendar	This part of the immunophenotyping of PBMC correlative study will be completed with the blood drawn as part of the CBCD.
Number and phenotype of PBMC including T & NK cells T and NK cell function	Cycle 1: W1D1 Cycle 1: W1D4 Cycle 1: W2D1 Cycle 1: W3D1 Cycle 2: W1D1 Cycle 2: W1D4 Cycle 3: W1D1 Cycle 5: W5D1 End of Study	**30mL**
Serum for PK, cytokine and immunogenicity analysis (Section 13.2). All collections are pre-dose unless otherwise specified. If a similar timepoint is listed in multiple sections, multiple analyses will be conducted on the 5mL collection.		**5mL**
PK	C1W1D1 C1W1D1 Post ALT-803 dose **(PHASE I ONLY)** 30 minute (± 5 minutes) from Time 0 2 hours (± 15 minutes) from Time 0 6 hours (± 60 minutes) from Time 0 24 hours(± 6 hours) from Time 0	
Serum Cytokine	C1W1D1 C1W1D4 C1W2D1 C1W3D1 C2W1D1 C2W1D4 C3W1D1 C5W5D1 EOS	
Immunogenicity	C1W1D1 C1W2D1 C1W3D1 C1W5D1 C2W3D1 C3W2D1 C4W1D1	

13.4. TUMOR BIOPSY STUDY (OPTIONAL)

Up to 20 patients will be selected for the tumor biopsy study.

Patients will be approached based on safety and feasibility of accessible tissue. Ultrasound and CT guided biopsy techniques may be employed to access tumor tissue, as well as biopsy of subcutaneous nodules which can be biopsied employing palpation of tumor for localization and biopsy. Bronchoscopy and surgery will not be employed to obtain biopsy

material. Specimen will be collected at baseline and at first restaging. Patients who do not make it to the first restaging time point will be replaced.

13.5. TUMOR TISSUE ANALYSIS

For all subjects, tissue from the diagnostic biopsy or previous surgery should be submitted for analysis if available. The list below outlines the FFPE list of prioritization. If possible, a block should be sent, but if the amount of tumor tissue available is limited, send what is available, starting with #1.

1.

10-15 unstained, positively charged, 5 micron thickness slides should be shipped according to instructions in the 102323 Correlatives Manual. Tissues should be labeled with the protocol number (102323), subject study ID. A separate biopsy will not be conducted for this tumor analysis study.

Samples will be sent to Adaptive Bioscience for genetic analysis for the presence of T cell-receptor.

14. DATA COLLECTION

Electronic CRF's will be provided for the recording of data and reporting that requires expedited review, such as SAE submissions. All data should be substantiated by clinical source documents organized within a patient research record. ICH Good Clinical Practices are to be followed. Data submission guidelines are outlined in the 102323 Operations Manual.

Electronic data for on study and follow-up patient data is submitted via the electronic system called REDCap. REDCap is managed from MUSC as a consortium partner under their CTSA. REDCap CRF is a secure, Web-based application designed to capture and manage research study data.

The system has been reviewed for 21CFR Part 11 compliance and has been deemed "21CFR 11 Capable." Users of the REDCap system are limited to members of the IRB approved research team who are delegated data management responsibilities, typically the study coordinator and data manager.

15. ADVERSE EVENT REPORTING REQUIREMENTS

The descriptions and grading scales found in the revised NCI CTCAE version 4.03 will be utilized for AE reporting. In addition, SAEs have special reporting requirements. AE and SAE criteria and reporting requirements are outlined in this section. For both serious and non-serious adverse events, the investigator must determine the severity of the event, "expectedness" of the event, and the relationship of the event to study treatment administration.

The study period during which all AEs must be reported begins after initiation of study treatment and ends at end of study treatment. All SAEs, including death due to disease progression, must be reported up to 30 days after the end of study treatment. After this period, investigators should only report AEs that are attributed to ALT-803. SAEs should be monitored until they are resolved or are clearly determined to be due to the patient's stable or chronic condition or intercurrent illness(es). Patients removed from study for unacceptable AE(s) will be followed for AE(s) for 24 weeks or until resolution or stabilization of the AE if that takes more than 24 weeks.

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately. Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to the study treatment, and actions taken. All AEs should be recorded and described in the AE database in REDCap.

15.1. PURPOSE

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. AEs are reported in a routine manner during a trial. Additionally, certain AEs must be reported in an expedited manner to allow for timelier monitoring of patient safety and care. The following guidelines prescribe routine and expedited adverse event reporting for this protocol.

Throughout the study, the Investigator will be required to provide appropriate information concerning any findings that suggest significant hazards, contraindications, side effects, or precautions pertinent to the safety of the study treatment under investigation.

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

15.2. DEFINITION OF ADVERSE EVENT

An AE will be considered any undesirable sign, symptom or medical or psychological condition **even if the event is not considered to be related** to the study treatment. Medical condition/diseases present before starting the study treatment will be considered adverse events only if they worsen after starting study treatment. An AE is also any undesirable and unintended effect of research occurring in human subjects as a result of the collection of identifiable private information under the research.
All toxicities, serious and non-serious that were not present at baseline will be reported in the REDCap AE database. Only clinically significant grade 3 or 4 abnormal lab

values that were not noted during the screening phase should be recorded; however, any clinical consequences of the abnormality, including dose modifications, regardless of grade, should be reported as AEs. There are special reporting requirements for injection site reactions (ISR). During the first 4 cycles, the 102323 side effect diary should be given to the patient to capture the information specific to ISRs as well as any other adverse events that may occur following ALT-803 administration.

Pre-existing diseases or conditions will not be considered AEs unless there is an increase in the frequency, duration or severity, or a change in the quality, of the disease or condition. Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) need not be considered AEs and should not be recorded as an AE. Progression of cancer also will not be considered an AE.

15.3. DEFINITION OF SERIOUS ADVERSE EVENT

An SAE is defined as any event that results in one of the following outcomes:

- death
- life threatening (places the subject at immediate risk of death)
- inpatient hospitalization or prolongation of existing hospitalization. Emergency room visits that do not result in admission to the hospital should be evaluated for one of the other serious outcomes.
- disability or permanent damage. Report is the event resulted in a substantial disruption of a person's ability to conduct normal life functions.
- congenital anomaly/birth defect.
- important medical important events. Report when the event does not fit the other outcomes, but the event may jeopardize the patient and may require medical or surgical intervention (treatment) to prevent one of the other outcomes. Examples include allergic bronchospasm (a serious problem with breathing) requiring treatment in an emergency room, serious blood dyscrasias (blood disorders) or seizures/convulsions that do not result in hospitalization. The development of drug dependence or drug abuse would also be examples of important medical events.

15.4. DEFINITION OF SEVERITY

AEs will be graded according to the revised NCI CTCAE v. 4. If toxicities are not defined, the intensity of each adverse event should be graded as follows:

GRADE 1	MILD: Sign or symptom noticeable, but does not interfere with normal daily activities.
GRADE 2	MODERATE: Sign or symptom sufficient to interfere with normal daily activities.
GRADE 3	SEVERE: Sign or symptom is incapacitating, with inability to perform daily activities.
GRADE 4	LIFE-THREATENING: sign or symptom poses immediate risk of death to this patient.

15.5. DEFINITION OF START DATE, STOP DATE AND DURATION

The definitions of AE start/stop dates and duration will be as follows:

Start Date	The date at which the AE is first noted
Stop Date	The date at which the AE is known to be resolved. If it is not known to have stopped, then indicate "ongoing."
Duration	A time in days

15.6. ACTIONS TAKEN

Action(s) taken may consist of the following:

None:	No actions taken
Discontinued test article:	Test article was permanently discontinued because of the AE
Change test article:	Test article was given at a lower dose, at a longer interval between doses, or was temporarily withheld because of the AE
Treatment:	Specified medication (to be listed on the concomitant medication chart) has been used as a countermeasure
Others:	Other actions, such as an operative procedure were required because of the AE

15.7. DEFINITION OF RELATIONSHIP TO STUDY TREATMENT

The categories for classifying the Investigator's opinion regarding the relationship of an AE to the study treatment are listed below.

Definitely related:	An adverse event occurring in a plausible time relationship to study treatment administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study treatment should be clinically plausible. The event must be definite pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary and feasible.
Possibly related:	An adverse event with a reasonable time sequence to administration of the study treatment, but which could also be explained by concurrent disease or other drugs or chemicals. Information on study treatment withdrawal may be lacking or unclear.
Not related:	An adverse event with a temporal relationship to study treatment administration which makes a causal relationship improbable, and in which other drugs, chemicals or underlying diseases provide plausible explanations.

15.8. EXPECTED ADVERSE EVENTS

Expected adverse events are those events known to be associated with study treatment. Expected adverse events are listed in the protocol, investigator brochure, package insert or

product label. Events should be classified as unexpected, expected or more prevalent than expected.

A table summarizing the expected events of ALT-803 is in Appendix D. Expected nivolumab events can be found in the nivolumab package insert.

15.9. NOTIFICATION OF SPONSOR OF SERIOUS ADVERSE EVENTS

The Investigator must notify the Sponsor-Investigator (via the SIS-Unit) of all SAEs **within 24 hours of first becoming aware of the event**. All SAE Reports should be submitted to the Sponsor-Investigator as outlined in the MUSC102323 Operations Manual.

Local IRB reporting requirements should be followed. Any IRB submissions and approvals or acknowledgments should be submitted to the SIS-Unit.

It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the subject's removal from treatment. If the subject was permanently withdrawn from the study or investigational product due to an SAE, this information must be included in either the initial or follow-up SAE Report Form.

The Investigator is required to comply with applicable regulations regarding the notification of his/her IRB.

16. ETHICAL AND REGULATORY CONSIDERATIONS

The following must be observed to comply with FDA regulations for the conduct and monitoring of clinical investigations; they also represent sound research practice.

16.1. INFORMED CONSENT

The investigator or other designated personnel will obtain written informed consent from all participating patients or their authorized representatives. The principles of informed consent are described by Federal Regulatory Guidelines (Federal Register Vol. 46, No. 17, January 27, 1981, part 50) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46). These federal guidelines as well as ICH GCP must be followed when obtaining informed consent.

Copies of the signed document will be given to the patient and filed in the Investigator's study file, as well as the patient's medical record if in conformance with the institution's Standard Operating Procedures.

16.2. INSTITUTIONAL REVIEW

This study must be approved by an appropriate institutional review committee as defined by Federal Regulatory Guidelines (Ref. Federal Register Vol. 46, No. 17, January 27, 1981, part 56) and the Office for Protection from Research Risks Reports: Protection of Human Subjects (Code of Federal Regulations 45 CFR 46)

The trial will not be initiated without approval of the appropriate IRB. All administrative requirements of the governing body of the institution will be fully complied with. This protocol, consent procedures, and any amendments must be approved by the IRB in compliance with current regulations of the FDA. A letter of approval will be sent to the institution(s) funding the study prior to initiation of the study and when any subsequent modifications are made. The IRB will be kept informed by the Investigator as to the progress of the study as well as to any serious or unusual adverse events.

16.3. DRUG ACCOUNTABILITY

For each drug supplied for a study, an accountability ledger containing current and accurate inventory records covering receipt, dispensing, and the return of study drug supplies must be maintained. Drug supplies must be kept in a secure, limited access storage area under the recommended storage conditions. These Accountability Forms must be readily available for inspection and are open to FDA or NCI inspection at any time.

16.4. PATIENT PRIVACY

In order to maintain patient confidentiality, all case report forms, study reports and communications relating to the study will identify patients by initials and assigned patient numbers. The FDA may request access to all study records, including source documentation for inspection. The Investigator/Institution will permit direct access to source data and documents by Altor, its designee, the FDA and/or other applicable regulatory authority. The access may consist of trial-related monitoring, audits, IRB, and FDA inspections.

Subject medical information obtained as part of this study is confidential, and must not be disclosed to third parties, except as noted below. The subject may request in writing that medical information be given to his/her personal physician.

Release of research results should preserve the privacy of medical information and must be carried out in accordance with Department of Health and Human Services Standards for Privacy of Individually Identifiable Health Information, 45 CFR 164.508.

16.5. PUBLICATION POLICY

The Investigators plan to publish and present the information obtained from the study.

16.6. RECORD RETENTION

The Sponsor-Investigator must ensure maintenance of all study records per applicable federal regulations.

17. MONITORING

The SIS-Unit will be responsible for the monitoring of study patient data and records; monitoring will be performed centrally. The SIS-Unit will be responsible for forwarding any applicable reports to the HCC DSMC for review.

The SIS-Unit will conduct patient eligibility audit reviews for all patients at each participating center prior to patient registration. During the course of the study, each site will be selected for an audit approximately twice a year. Audits will be conducted remotely. During Phase I, progress reports will be submitted in conjunction with the end of each cohort. During Phase II, progress reports will be submitted to the HCC DSMC approximately twice a year.

Details regarding the monitoring and auditing process are outlined in the MUSC 102323 Operations manual.

The SIS-Unit will maintain a shadow regulatory binder for all participating centers. Required regulatory documents, including those needed for site initiation, during activation, and study closure, are outlined in the MUSC 102323 Operations Manual for this study.

The SIS-Unit will provide regulatory documents and patient data upon reasonable request to Altor Bioscience, Inc.

17.1. PROTOCOL DEVIATIONS

For the purposes of this study, a **protocol deviation** is any variance from the protocol involving a subject or subjects that is not approved by the IRB prior to its initiation or implementation, and occurs when a member of the study team departs from the IRB-approved protocol in any way without the investigator first obtaining IRB approval. Protocol Deviations have special reporting requirements.

Any protocol deviation and any supporting documentation will be submitted to the SIS-Unit by the site within 10 days of notification as outlined in the IIT SOP for this study. Local IRB reporting requirements should be followed. Any IRB approvals or acknowledgments should be submitted to the SIS-Unit.

17.2. DATA SAFETY MONITORING BOARD

The HCC DSMC will have oversight of the protocol. The HCC DSMC will meet at a minimum on a semi-annual basis to review the audits and progress reports for this IIT.

In addition, all protocol deviations and SAEs as defined above will be reviewed by the HCC DSMC at monthly meetings. As new protocol deviations or serious adverse events are reported to the SIS-Unit, the SIS-Unit will review these reports for form completion and follow up if more information is warranted. The SIS-Unit will forward the event report to the HCC DSMC so that the information can be reviewed at the next available DSMC meeting. During the DSMC review, the DSMC can make recommendations for any further study action. The SIS-Unit will maintain a copy of the DSMC approval letters for each event reviewed for this study within the site's central study file and will distribute to the participating site, if applicable.

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APPENDIX A: ECOG PERFORMANCE STATUS SCALE

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints.
1	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).	90	Able to carry on normal activities; minor signs or symptoms of disease.
		80	Normal activity with effort
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.	70	Cares for self; unable to carry on normal activity or to do active work.
		60	Requires occasional assistance but is able to care for most of his/her needs.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	50	Requires considerable assistance and frequent medical care.
		40	Disabled, requires special care and assistance.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	30	Severely disabled, hospitalization indicated. Death not imminent.
		20	Very sick, hospitalization indicated. Active supportive treatment necessary.
		10	Moribund.
5	Dead.	0	Dead.

APPENDIX B: CALCULATION OF CREATININE CLEARANCE

Estimation of creatinine clearance using Cockroft and Gault method:

Cl_{CR} for males (mL/min) = $\frac{[140 - \text{age (years)}] \times [\text{weight (kg)}]}{(72) \times [\text{Serum creatinine (mg/dL)}]}$

Cl_{CR} for females (mL/min) = $\frac{(0.85) \times [140 - \text{age (years)}] \times [\text{weight (kg)}]}{(72) \times [\text{Serum creatinine (mg/dL)}]}$

For SI units:

Cl_{CR} for males (mL/min) = $\frac{[140 - \text{age (years)}] \times [\text{weight(kg)}] \times (1.23)}{[\text{Serum creatinine (}\mu\text{mol/L)}]}$

Cl_{CR} for females (mL/min) = $\frac{[140 - \text{age(years)}] \times [\text{weight(kg)}] \times (1.05)}{[\text{Serum creatinine (}\mu\text{mol/L)}]}$

Calculation of creatinine clearance based on 24-hour urinary creatinine excretion and concurrent serum creatinine levels:

$$Cl_{CR} = \frac{C_U \cdot V}{C_{CR}}$$

Here, C_U is the concentration of creatinine in the urine (mg/dL or µmol/L, for SI units), V is the urine volume (in mL per minute of urine produced during the collection period), Cl_{CR} is the serum creatinine concentration (mg/dL or µmol/L, for SI units), and Cl_{CR} is the creatinine clearance in mL per minute.

APPENDIX C: NYHA CLASSIFICATION OF CONGESTIVE HEART FAILURE

Class I	Subjects with no limitation of activities; they suffer no symptoms from ordinary activities
Class II	Subjects with slight, mild limitation of activity; they are comfortable with rest or with mild exertion.
Class III	Subjects with marked limitation of activity; they are comfortable only at rest.
Class IV	Subjects who should be at complete rest, confined to bed or chair; any physical activity brings on discomfort and symptoms occur at rest.

APPENDIX D: ALT-803 – EXPECTED ADVERSE EVENTS

CTCAE v4.0 System	ADVERSE EVENT TERMS
BLOOD AND LYMPHATIC SYSTEM DISORDERS	Disseminated intravascular coagulation
	Hemoglobin (anemia)
	Hemolysis
	Infection (documented) with grade 1 or 2 neutropenia
	Infection (documented) with grade 3 or 4 neutropenia
	Intravascular coagulopathy
	Thrombotic microangiopathy
CARDIAC DISORDERS	Cardiac arrest
	Coronary artery disorder
	Endocarditis
	Myocarditis (CHF)
	Myocardial ischemia/infarction
	Pericardial effusion
	Pericarditis (ECG changes)
	Supraventricular (atrial) tachycardia, atrial fibrillation
	Ventricular tachycardia
EYE DISORDERS	Mydriasis
	Permanent or transient blindness secondary to neuritis
	Pupillary disorder
GASTROINTESTINAL DISORDERS	Abdomen enlarged
	Abdominal pain
	Bowel perforation/infarction, necrosis
	Colitis
	Constipation
	Diarrhea/ Bloody diarrhea
	Duodenal ulceration
	Heartburn
	Hemorrhage/GI
	Hemorrhage/nose
	Intestinal obstruction/ Ileus
	Mucositis/Stomatitis (Pharynx)
	Nausea
	Pancreatis
	Tracheo-esophageal fistula
	Vomiting
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	Edema (peripheral)
	Fatigue (asthenia, malaise, lethargy)
	Fever, sweating
	Hemorrhage/other
	Pain NOS (not otherwise specified)
	Rigors/chills
HEPATOBILIARY DISORDERS	Cholecystitis
IMMUNE SYSTEM DISORDERS	Allergic reaction

CTCAE v4.0 System	ADVERSE EVENT TERMS
INFECTIONS AND INFESTATIONS	Infection without neutropenia
	Infection with unknown ANC
	Meningitis
	Sepsis
INVESTIGATIONS	Alkaline phosphatase increase
	Amylase increase
	Anuria/Oliguria
	Creatinine increase
	Hyperbilirubinemia
	Leukopenia, Lymphopenia
	Neutropenia
	SGOT/SGPT increase
	Thrombocytopenia
	Weight gain, weight loss
METABOLISM AND NUTRITION DISORDERS	Acidosis
	Anorexia
	BUN increase
	Electrolyte imbalance
	Hyperuricemia
	Hypoalbuminemia
	NPN increase
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	Arthritis (non-specific)
	Fibrosis
	Muscle weakness, generalized or specific area (not due to
	Soft tissue necrosis
NERVOUS SYSTEM DISORDERS	Coma
	Dizziness
	Edema (cerebral)
	Encephalopathy
	Encephalopathy
	Headache
	Hemorrhage/CNS
	Neuropathy
	Seizure
	Somnolence
	Stroke
	Stupor
	Taste alteration (dysgeusia)
PSYCHIATRIC DISORDERS	Agitation
	Confusion
	Insomnia
	Mood Alteration (anxiety)
	Paranoid reaction
	Psychosis
	Severe depression leading to suicide
RENAL AND URINARY DISORDERS	Acute tubular necrosis
	Nephritis
	Proteinuria

CTCAE v4.0 System	ADVERSE EVENT TERMS
	Renal failure
	Renal function abnormal
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	ARDS
	Cough increase
	Dyspnea
	Edema (pulmonary)
	Hemoptysis
	Hemorrhage/pulmonary
	Hypoventilation
	Hypoxia
	Intubation
	Lung disorder (pulmonary congestion, rales, rhonchi)
	Pneumothorax
	Pulmonary infiltration
	Respiratory arrest
	Respiratory failure
	Rhinitis
	Unspecific pulmonary changes
SKIN AND SUBCUTANEOUS TISSUE DISORDERS	Hand-foot skin reaction
	Hair loss/alopecia (scalp or body)
	Pruritus/Itching
	Rash/Dermatitis
	Rash/Desquamation
	Rash/Erythema multiform
	Urticaria, Flushing
VASCULAR DISORDERS	Hypotension
	Thrombosis/embolism
	Vasodilation