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PHASE I TRIAL OF ALT-803, A NOVEL RECOMBINANT INTERLEUKIN-15 COMPLEX, IN PATIENTS WITH ADVANCED SOLID TUMORS

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Abstract

Background: IL-15 induces the activation and proliferation of NK and memory CD8+ T cells and has preclinical antitumor activity. Given the superior activity and favorable kinetics of ALT-803 (IL-15N72D:IL-15R α Su/IgG1 Fc complex) over recombinant human IL-15 (rhIL-15) in animal models, we performed this first-in-human Phase I trial of ALT-803 in patients with advanced solid tumors.

Methods: Patients with incurable advanced melanoma, renal cell, non-small cell lung, and head and neck cancer were treated with ALT-803 0.3–6 μ g/kg weekly i.v. or 6–20 μ g/kg weekly s.c. for 4 consecutive weeks, every 6 weeks. Immune correlates included pharmacokinetics, immunogenicity, lymphocyte expansion and function. Clinical endpoints were toxicity and antitumor activity.

Results: Twenty-four patients were enrolled; eleven received i.v. and 13 received s.c. ALT-803. Of these patients, 9 had melanoma, 6 renal, 3 head and neck, and 6 lung cancer. Although total

lymphocyte and CD8⁺ T cell expansion were modest, NK cell numbers rose significantly. Neither anti-ALT-803 antibodies nor clinical activity were observed. Overall, ALT-803 was well-tolerated, with adverse effects including fatigue and nausea most commonly with i.v. administration, while painful injection site wheal was reported most commonly with s.c. ALT-803.

Conclusions: Subcutaneous ALT-803 produced the expected NK cell expansion and was well-tolerated with minimal cytokine toxicities and a strong local inflammatory reaction at injection sites in advanced cancer patients. These data, together with compelling evidence of synergy in preclinical and clinical studies, provide the rationale for combining ALT-803 with other anti-cancer agents.

Keywords

Interleukin-15; ALT-803; IL-15 superagonist; clinical trial; immunotherapy

INTRODUCTION

IL-15 is one of 6 known common gamma chain (γ c) cytokines that activate T cells and natural killer (NK) cells. The IL-15 receptor shares both the β and γ chains with the IL-2 receptor, leading to similar effects on the stimulation and expansion of memory CD8⁺ T cells and NK cells. However, these two cytokines have important differences conferred by their distinct α receptor structures and distribution. While the IL-2 α receptor chain is associated with shared β and γ chains on target lymphocytes, the membrane-associated IL-15 α receptor on antigen-presenting cells (APC) binds the IL-15 molecule and presents it to the $\beta\gamma$ c receptors located on the surface of T and NK cells. Functional differences include expansion and maintenance of regulatory T cells (Treg) by IL-2 but not IL-15; activation-induced cell death stimulated by IL-2 but not by IL-15; maintenance of memory CD8⁺ T cells by IL-15 but not by IL-2; and a major role in controlling the lymphocyte pool size, including reconstitution following lymphodepleting therapy, by IL-15 but not IL-2 [1].

IL-15 has shown antitumor activity as a single agent as well as in combinations with therapeutic antibodies or immune checkpoint-blocking antibodies in animal models [2, 3]. However, IL-15 alone is less likely to mediate favorable immunomodulatory effects, since it is trans-presented to CD8⁺ T and NK cells in a complex with the IL-15 α receptor by APCs. Further, the unmodified recombinant IL-15 molecule has a short serum half-life and, when administered intravenously (i.v.), can cause inflammatory and hemodynamic toxicities similar to those of IL-2 [4], which is now used rarely for cancer therapy due to its unfavorable therapeutic ratio. More recently, IL-15 has been developed as a therapeutic immune complex in association with the IL-15 α receptor or with a non-neutralizing anti-IL-15 antibody [5]. ALT-803 is a complex containing 2 molecules of an optimized amino acid-substituted (N72D) IL-15 “superagonist”, 2 molecules of the IL-15 α receptor “sushi” domain fused to a dimeric human IgG1 Fc that confers stability and prolongs the half-life of the overall complex (IL-15N72D:IL-15R α Su/IgG1 Fc complex).

ALT-803 has been tested for activity, immunogenicity, pharmacokinetics and immunomodulatory effects in mice bearing various tumors and shown to have great promise in melanoma, myeloma and urothelial cancers [6]. Persistence of ALT-803 in lymphoid

organs up to 70 hours and detection of *in vivo* NK and/or T cell stimulatory effects for as long as 7 days prompted initial dosing of i.v. ALT-803 weekly in humans [6, 7]. Later murine studies showing 8-fold lower C_{max} with s.c. vs. i.v. administration, suggesting a decreased likelihood of cytokine-related systemic symptoms with s.c. dosing, as well as evidence of comparable immunostimulatory effects [7, 8], made the out-patient s.c. route of administration attractive for evaluation in patients.

This current Phase I study was one of two first-in-human studies of ALT-803, with the other trial conducted in patients with hematologic malignancy in relapse following allogeneic hematopoietic cell transplant [9]. We analyzed the effects of i.v. or s.c. administered ALT-803 to patients with advanced solid tumors who had failed standard therapy for their malignancies. The 5 eligible tumor types (melanoma, renal cancer, non-small cell lung cancer, head and neck squamous cell cancer and sarcoma) were chosen for their potential benefit from immunomodulatory therapy. The immune correlates evaluated included expansion of lymphocyte subsets and other functional responses, pharmacokinetics and immunogenicity of the ALT-803 complex.

MATERIALS AND METHODS

Subjects

Patients eligible for this study were adults over 18 with melanoma, renal cell, non-small cell lung, head and neck cancer, or sarcoma, who had previously received standard therapy regimens with adequate washout time from possible toxicities, and were unlikely to benefit from other disease-directed therapies. Requirements included hemoglobin at least 10 g/dL, total white blood cell (WBC) count at least 3000/ μ L, absolute lymphocyte count (ALC) at least 500/ μ L, absolute neutrophil count (ANC) at least 1000/ μ L, platelets at least 100,000/ μ L, serum bilirubin and creatinine within institutional normal limits, and serum hepatic transaminases \leq 2.5 times the institutional upper limits of normal. Patients were excluded if they had chronic obstructive pulmonary disease, active cardiac or other major illness or active brain metastasis, pregnancy, serologic markers of active hepatitis B or C infection, or dependence on therapeutic doses of steroids (replacement doses were permitted for patients previously treated with immune checkpoint inhibitors developing adrenal insufficiency).

Study design

The trial was coordinated and monitored by the Cancer Immunotherapy Trials Network (CITN) and conducted at 5 clinical centers in the United States (University of Washington/Seattle Cancer Care Alliance; University of Minnesota; Rutgers University; Cleveland Clinic Foundation; Dartmouth-Hitchcock Medical Center) between 5/2014 and 7/2017. The trial was approved and monitored by the Cancer Therapy Evaluation Program (CTEP) as well as the investigational agent sponsor, Altor BioScience (Miramar, FL), who held the IND. The Melanoma Research Alliance provided additional funding (A72030). The trial was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines. Regulatory approval was obtained centrally through the Fred Hutchinson Cancer Research Center Institutional Review Board

(IRB) or the local IRB at participating centers, and all patients provided their written, voluntary informed consent.

The study was designed with the primary objective of identifying an optimal dose of ALT-803, defined by either dose-limiting toxicity or by a minimal effective dose associated with lymphocyte expansion. The NCI Common Toxicity Criteria version 4 (CTCAE4) was used to classify adverse events, and their potential attribution to the study drug was determined by the local principal investigator and reviewed during biweekly teleconferences of the protocol committee (CITN), a representative of the NCI, and a representative of the drug sponsor, Altor BioScience. Clinical responses were assessed using the RECIST 1.1 criteria. Data and safety monitoring were assured by this high-frequency communication with all members of the research team.

In this Phase I dose-escalation trial, similar rules were applied to patients receiving ALT-803 by either route of administration: after the first two patients treated at the two lowest i.v. dose levels passed the safety assessments, 3 patients were then treated at each dose level using a modified Fibonacci dose-escalation scheme. If a DLT occurred in one of the first 3 patients enrolled at a dose level, the cohort size was expanded to 6 patients. If 2 out of 3 or 6 patients experienced DLTs, dose escalation would be halted and the prior level considered the MTD. DLTs were defined, based on previous clinical studies with rhIL-15, as any grade 3 toxicity except for the following: grade 3 fatigue or anorexia; grade 3 hypotension; grade 3 hypocalcemia, hypokalemia, hypomagnesemia, hyponatremia, hypophosphatemia that responded to medical intervention; fever of $<41^{\circ}\text{C}$ for <12 hours; grade 3 or 4 nausea; vomiting or diarrhea <72 hours; grade 3 injection site reaction not requiring operative intervention. Hematologic exceptions were grade 3 or 4 transient lymphopenia, grade 3 or 4 transient neutropenia, febrile neutropenia not requiring urgent intervention and grade 3 lymphocyte increase. $\text{ALC} > 25,000/\mu\text{L}$ was also not considered a DLT but was designated the “maximum desired effect” and would prompt interruption of treatment until the lymphocyte count dropped without precluding additional subsequent treatment. However, $\text{ALC} > 35,000/\mu\text{L}$ was considered a DLT.

Investigational agent

ALT-803 is a soluble complex consisting of 2 human IL-15 variant (N72D) molecules bound to a dimeric IL-15R sushi domain/human IgG1 Fc fusion protein produced in Chinese hamster ovary cells [10, 11]. Apart from the N72D substitution, all protein sequences are human.

The dose of ALT-803 was based on actual body weight and administered i.v. or s.c. weekly for 4 consecutive weeks, followed by a 2 week rest period during which patients continued to be monitored for toxicities and underwent tumor reassessment for therapeutic outcome. All patients were intended to receive two 6-week cycles of therapy, but patients who experienced disease progression (or excessive toxicity) were removed from treatment and monitored for an additional 30 days for delayed toxicity. Treatment of the second and third patient in each dose level cohort could begin only after the previous patient in the same dose level had been observed for safety over the entire first 4-week treatment cycle. Subcutaneous

ALT-803 was administered by an experienced research nurse. In some cases, the total dose was divided into 2–3 injections due to volume.

Clinical and Investigational Assessments

Complete Blood Counts—CBC data, including total white blood cell and calculated absolute lymphocyte counts were obtained from individual subjects' local labs using samples obtained just prior to the first dose (Day 1) as well as 12 and 24 hours later, and Days 4, 8, 15, 22, and 29 of Cycle 1 and Days 1, 4, 15 and 29 of subsequent cycles. On dosing days, blood samples were obtained prior to ALT-803 administration.

Specimen handling and processing—Heparinized whole blood samples collected at each clinical site were shipped by overnight express mail in insulated shippers that contained LogTag temperature recorders (Northcote, Auckland, New Zealand) to continuously record ambient temperatures during shipment. Samples were received at the University of Washington CITN Central Laboratory an average of 34 hours later. Aliquots of fresh whole blood were used for real-time antibody labeling for flow cytometric analyses and the remainder of the samples processed to plasma and PBMC using standard Ficoll-Hypaque isolation immediately upon receipt. PBMC were cryopreserved in 10% DMSO (Sigma, St. Louis, MO) and 12.5% HSA (Gemini, Atlanta, GA) at -80°C and subsequently maintained in vapor phase liquid nitrogen freezers. Serum was collected at the clinical sites within 4 hours of blood draw and frozen at -80°C . Batched samples were later shipped on dry ice to the CITN Central Laboratory and then subsequently to Altor BioScience for testing.

Immunophenotyping—Whole blood flow cytometric analyses were performed initially using fresh blood samples from Day 1, 8, 15, 22 and 29 time points. The protocol was amended in April 2015 to add flow cytometric testing on Day 4 of each cycle. Fresh whole blood samples were labeled with fluorescently-labeled antibodies to cell surface molecules CD45 (2D1), CD3 (UCHT1), CD8 (SK1), CD56 (NCAM16.1), CD16 (3G8), CD14 (MOP9), CD123 (9FS) (all BD Biosciences, San Jose, CA) and CD4 (RPA-T4), CD19 (HIB19), and HLA-DR (L243) (all Biolegend, San Diego, CA) after overnight shipping to the CITN Central Lab, using a method adapted from Hensley et al [12]. Samples were treated with BD FACSTTM Lysing Solution (BD Biosciences) and immediately frozen at -80°C for later batch testing on a BD LSRII flow cytometer. Absolute cell numbers were obtained using Trucount tubes (BD Biosciences). Presence of intracellular Ki-67 was analyzed using thawed PBMC labeled with antibodies to CD14 (MoP9, exclusion marker), CD56 (NCAM16.2), CD4 (SK3), CD8 (SK1) (all, BD Biosciences), CD3 (SK7), Ki67 (both, Biolegend) as well as Fixable Viability Dye eFluor 780 and FoxP3/Transcription Factor Staining Buffer Set (both eBioscience, San Diego, CA). Data analysis was performed using FlowJo software (Treestar, Ashland, OR).

Pharmacokinetic (PK) and Immunogenicity Analyses

Blood samples were collected from subjects before, and 30 minutes, 2, 4, 8, and 24 hours after the first dose of ALT-803 given i.v. or s.c. during Cycle 1, from the first two patients of each dose cohort according to study protocol. Serum was frozen at local sites at -20°C to -80°C and shipped in batches on dry ice to the Central CITN Laboratory. Samples were

subsequently shipped to Altor BioScience on dry ice for batch ELISA testing for ALT-803 pharmacokinetic and immunogenicity analyses.

Serum ALT-803 concentrations were assessed using a qualified assay at Altor BioScience, utilizing a human IL-15 specific ELISA kit (R & D Systems, Minneapolis, MN) and ALT-803 for generation of the standard curve. Mean values of triplicate wells are reported.

An ELISA developed by Altor BioScience was used to detect anti-ALT-803 antibodies in serum obtained prior to dosing on Cycle 2 Day 1 (C2D1, baseline), and at the 14 Day follow-up (14D FU). This qualified anti-ALT-803 bridging ELISA uses ALT-803 as the capture reagent and HRP-conjugated ALT-803 for detection. Samples were assayed in triplicate in a dilution series (1:100–1:6400) and considered positive for anti-ALT-803 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.

RESULTS

Subject characteristics and drug administration

Demographic data, treatment dose level and duration of therapy for all study subjects are shown in Table 1. Eleven patients received i.v. ALT-803, with a single patient at each of the two lowest doses 0.3 and 0.5 µg/kg, for this first-in-human study. Three patients were treated at each subsequent dose of 1, 3, and 6 µg/kg/dose. To simplify delivery of this agent on an out-patient basis and based on additional preclinical data from Altor BioScience [8], the route of administration was changed to subcutaneous after the initiation of this study. A total of thirteen patients were then treated with s.c. dose levels of 6, 10, 15 and 20 µg/kg/dose (3 patients each at 6, 10 and 20 µg/kg and 4 patients at 15 µg/kg). Note that Subject 19 is considered part of the 15 µg/kg dose cohort, although she received only one 15 µg/kg dose of ALT-803; after experiencing transient grade 4 neutropenia, she was treated at a decreased dose of 10 µg/kg. Over both i.v. and s.c. dose cohorts, 9 patients had melanoma, 6 renal cancer, 3 head and neck squamous cell cancer and 6 non-small cell lung cancer.

Eleven of 24 subjects receiving i.v. or s.c. ALT-803 completed one 6-week cycle of therapy; 7 subjects completed 2 cycles; 2 completed 3 cycles; and 4 subjects completed <1 cycle, of whom 3 discontinued for disease progression (Table 1). Consistent with a Phase I clinical trial, no clinical or radiologic complete or partial responses occurred. Overall, two patients withdrew consent, two patients discontinued therapy at the discretion of the treating physician, and the remaining 20 patients discontinued therapy for disease progression.

Toxicities of treatment

Toxicities were generally mild and non-dose-limiting throughout the dose ranges for both i.v. and s.c. routes of administration (Table 2), except for Subject 19, whose dose was reduced for grade 4 neutropenia. For simplicity, only adverse events reported for 3 or more subjects are shown in Table 2. The toxicity profile for the i.v. dose cohorts was low grade (1–2) and consistent with cytokine administration: fatigue, nausea, vomiting, chills and fever, in decreasing order of frequency. No patient required prolongation of hospitalization (first-dose stay) or hospital admission (subsequent doses). Hypotension was not reported in

the i.v. dose cohorts, and was described in only 2 subjects in the s.c. dose cohorts (one 15 µg/kg subject and one 20 µg/kg subject), both grade 1. For the s.c. dose cohorts, however, the most common adverse event was injection site reaction, occurring in 11 of 13 subjects. These were described as a large wheal around the prior injection site with onset at approximately 3 days, peak intensity at 5 days, and resolution by 7 days following injection. No recall effects were reported at the original site following subsequent injection at different sites. Biopsy from one subject demonstrated the presence of an intense perivascular lymphomononuclear infiltrate consisting primarily of CD68+ macrophages and CD3+ lymphocytes (nearly equal percentages of CD4+ and CD8+ subsets), with rare CD20+ B and CD56+ NK/NKT cells. Similar results were reported in two recent trials using ALT-803 as single agent or in combination with a checkpoint inhibitor [9, 13].

One patient with lung cancer (Subject 24) received 1 cycle of ALT-803 at the highest dose of 20 µg/kg SC, had a normal pre-study cardiac evaluation and went off therapy for progression. One month after the last ALT-803 dose, the patient presented with congestive heart failure, and studies revealed a left ventricular ejection fraction of 12% with diffuse, global myocardial dysfunction and 4-chamber dilated cardiomyopathy, not consistent with an ischemic etiology. MRI findings did not suggest inflammation. Nevertheless, the patient was treated empirically with glucocorticosteroids, and experienced clinical improvement. Myocardial biopsy demonstrated fibroblast proliferation suggestive of a subacute process but no histologic evidence of acute inflammatory infiltrate. The event was considered a grade 4 serious adverse event that was unlikely to be directly related to ALT-803; however, since a clear alternative mechanism could not be identified and the patient improved with immunosuppression, the possibility of a contribution by ALT-803 could not be ruled out.

Pharmacokinetics, immunogenicity and pharmacodynamics

Pharmacokinetic data were obtained pre-dose and at 30 minutes, 2, 4, 8 and 24 hours after ALT-803 dosing, from 2 subjects in each dose cohort according to the study protocol (Figure 1). The time to maximum serum concentration (T_{max}) occurred consistently at 30 minutes after i.v. administration. By contrast, subcutaneous ALT-803 exhibited a very gradual increase in serum concentration, with maximal serum levels >100-fold less than the average peak after i.v. ALT-803. In 4 of 8 s.c. subjects, the serum concentration peaked at 4 to 8 hours, but in the remaining 4 subjects, the highest concentration measured occurred at 24 hours, the last time point tested. Therefore, it is possible that the T_{max} occurred either at or after 24 hours in these 4 subjects.

Immunogenicity was tested on samples collected at baseline, prior to dosing on Cycle 2 Day 1 (C2D1), and at the 14 Day follow-up (14D FU) time point by Altor BioScience, as described. Eight of 11 and 8 of 13 subjects treated with i.v. and s.c. ALT-803, respectively, were tested at the 14D FU time point; and 1 and 3 subjects were negative at C2D1 but not tested at 14D FU. No reactivity was detected in any sample (data not shown).

In addition to safety, the white blood cell (WBC) count and calculated absolute lymphocyte count (ALC) were primary endpoints for this phase I trial which sought to evaluate the impact of ALT-803 on the expansion of IL-15-responsive lymphocytes. ALT-803 demonstrated little effect on overall WBC counts when maximal counts during Cycle 1 were

compared to baseline counts (s.c. range 1.0–1.3-fold increase, Figure 2A, i.v. range 1.1–1.3-fold increase, Supplemental Figure 1A). The ALC transiently decreased after the first dose of ALT-803, reaching a nadir between 12 hours and 4 days post-dose for s.c. dosing (Figure 2B) and 12–24 hours for i.v. dosing (Supplemental Figure 1B). Subsequently, the ALC recovered but in most cases did not rise much higher than baseline over the course of Cycle 1 for the s.c. dose cohorts, with the exception of a maximal 2.1-fold increase occurring in the 15 µg/kg dose cohort, peaking 1–2 weeks after the last dose of ALT-803 during Cycle 1. The mean maximal post-treatment ALC increases during Cycle 1 were 1.2, 1.3, 2.1, and 1.1-fold respectively for the four s.c. dose levels of 6 µg/kg (N=3), 10 µg/kg (N=3), 15 µg/kg (N=4), and 20 µg/kg/dose (N=3). It is worth noting that Subject 21, included in the 15 µg/kg dose cohort, had her dosing decreased after one dose to 10 µg/kg through 3 cycles (the remainder of her participation in the study). For the four i.v. dose cohorts, the mean maximal post-treatment ALC increases during Cycle 1 were 1.1, 1.4, 1.5, and 1.6-fold respectively for the 0.3 & 0.5 µg/kg (N=1 each), 1 µg/kg (N=3), 3 µg/kg (N=3), and 6 µg/kg/dose (N=3), with peaks occurring slightly later, after Day 22.

Serial flow cytometric analyses of NK cells (CD3-/CD56+) and NK cell subsets (defined by level of CD56 expression) revealed a dip in circulating NK cell counts at the earliest time point tested after the first dose (Day 4) for the s.c. dose cohorts, as was observed for the ALC, but not for the i.v. dose cohorts. In contrast to the ALC response, however, substantial increases in circulating total NK cell numbers were found after ALT-803 treatment, in both the i.v. (Supplemental Figure 2) and s.c. dose cohorts (Figure 3), with maximal levels typically occurring in Cycle 1 between days 15 and 29. Among the eight subcutaneously-treated patients who continued treatment beyond the first cycle, 6 had evaluable data, and circulating total NK cell counts did not rise beyond the levels attained during Cycle 1. The mean maximal post-treatment total NK cell increases during Cycle 1 were 2.6, 2.3, 7.9 and 3.3-fold respectively for the four s.c. dose levels of 6 µg/kg (N=3), 10 µg/kg (N=3), 15 µg/kg (N=2), and 20 µg/kg/dose (N=3). Only 2 subjects from the 15 µg/kg s.c. dose cohort had useable flow cytometry data available (Figure 3B), and one of these two subjects had a dramatic response to ALT-803 (13.5-fold increase in NK cell numbers). Thus the dramatic increase in mean fold change for the 15 µg/kg dose cohort can be explained by the unusually high response in Subject 21 and the small sample size for this dose cohort. For the i.v. dose cohorts, the mean maximal post-treatment total NK cell increases during Cycle 1 were more modest at 1.2, 1.4, 2.1, and 2.5-fold respectively for the 0.3 & 0.5 µg/kg (N=1 each), 1 µg/kg (N=3), 3 µg/kg (N=3), and 6 µg/kg/dose cohorts (N=3).

Both circulating CD56^{bright} and CD56^{dim} NK cells trended together, although mean maximal fold-increases were greater for the smaller CD56^{bright} subpopulation in the s.c. dose cohorts (3.1, 1.8, 13.3, and 6.3-fold for 6 µg/kg, 10 µg/kg, 15 µg/kg, and 20 µg/kg respectively) compared to the larger CD56^{dim} subpopulation (2.6, 2.2, 7.6, and 2.7-fold for 6 µg/kg, 10 µg/kg, 15 µg/kg, and 20 µg/kg respectively, Figure 3C, D). Similarly, for the highest i.v. dose cohort of 6 µg/kg/dose, the CD56^{bright} subpopulation demonstrated a greater fold increase (5.3-fold) compared to the CD56^{dim} NK cells (2.6-fold, Supplemental Figure 2). This finding is not surprising, as CD56^{bright} NK cells are known to proliferate at a higher rate than CD56^{dim} NK cells, and this phenomenon has been described for other IL-15 agents [14].

By contrast, overall circulating CD4+ and CD8+ T cell numbers (including CD8+ memory T cells) did not increase substantially with ALT-803 treatment; overall means were 1.1 and 1.3-fold increases respectively for s.c. and 1.3 and 1.5-fold increases respectively for i.v. dose cohorts during Cycle 1. Subject 20 (15 µg/kg s.c.) uniquely exhibited a 3.6-fold increase in CD25+CD127^{neg} Treg cell numbers by Cycle 2 Day 1, but no increase in Treg cell numbers was observed for the remaining 6 subjects with available data (mean 1.1-fold increase).

Interestingly, intracellular analyses with a fluorescently labeled antibody specific for Ki-67, a protein present only during active phases of the cell cycle but not quiescence, demonstrated evidence of proliferation in the CD8+ T cell compartment, with lower levels in the CD4+ T cell compartment at Days 4 and 8 after ALT-803 administration (Figure 4A, B). Subject 21 (15 µg/kg s.c.) exhibited a remarkable response to ALT-803, with 63 and 9.8-fold increases in Ki-67 expression among CD8+ and CD4+ T cells, respectively. After exclusion of this outlier, the mean maximal fold increases among CD8+ and CD4+ T cells were similar at 10 and 3-fold for the 15 µg/kg and 8.1 and 2.9-fold for the 20 µg/kg dose cohorts, respectively. Consistent with these results, after s.c. ALT-803 dosing, a larger fraction of CD8+ T cells demonstrated expression of HLA-DR, a human T cell activation marker (mean 3.1, 2.7, 6.6, and 4.7-fold increases for the 6 µg, 10 µg, 15 µg, and 20 µg/kg dose cohorts, respectively), and to a lesser degree on CD4+ T cells (mean 1.6, 1.6, 3.0, and 1.6-fold increases for the 6 µg, 10 µg, 15 µg, and 20 µg/kg dose cohorts). The highest level of intracellular Ki-67 expression occurred on either Days 4 or 8 after the first dose of ALT-803, while maximal expression of HLA-DR on CD8+ T cells occurred consistently on Day 4 (with only one exception at Day 8). Increases in PD1-expressing CD8+ T cells were also found on Day 4, smaller than, but consistent with increased HLA-DR expression (data not shown). More modest increases in HLA-DR expressing T cell percentages were seen in the i.v. dose cohorts for both CD4+ (range 1.2–1.5-fold change) and CD8+ (range 1.2–2.3-fold change) T cells (Supplemental Figure 3). Interestingly, most of these increases were maximal later during Cycle 1 in the i.v. compared to the s.c. dose cohorts, with 9 of 11 subjects exhibiting maximal increases between Days 15 and 29 (data not shown).

DISCUSSION

We report the safety, tolerability, pharmacokinetics, and immunologic effects of ALT-803 administered intravenously (0.3 – 6 µg/kg/dose) or subcutaneously (6 – 20 µg/kg/dose) in patients with advanced tumors. The adverse events observed in this study were generally mild for patients treated with either i.v. or s.c. ALT-803. Despite local injection site reactions occurring in 85% of s.c. patients, a formally-defined maximum tolerated dose was not identified. As often found in phase I studies, single-agent clinical benefit for ALT-803 was not observed; our study population was comprised of adults with several types of advanced solid tumors who had failed to benefit from other therapies. Both i.v. and s.c. ALT-803 led to increases in circulating NK cells, with the greatest fold increases occurring in the CD56^{bright} NK cell subpopulation. While circulating numbers of CD8+ T cells did not increase substantially, Ki-67 analyses indicated significant proliferation of these cells concurrent with increased HLA-DR expression occurring within a week of the first dose.

The pharmacokinetics of ALT-803 were understandably different between the i.v. and s.c. dose cohorts. Intravenous administration led to maximum serum concentrations (C_{\max}) >100-fold compared to s.c., and occurred consistently early for the i.v. dose cohorts (30 minutes post-dose) compared to between 8 and 24 hours for s.c. administration, reflecting the slower and more variable absorption of ALT-803 via the latter route. The low ALT-803 C_{\max} may also explain the mild systemic toxicities experienced by patients in the s.c. dose cohorts.

Although total WBC and ALC levels were not very informative, more detailed flow cytometric analyses demonstrated increases in circulating NK cells for both i.v. and s.c. ALT-803-treated groups, similar to results from a phase 1 trial of s.c. rhIL-15 alone delivered by daily injection [14]. Moreover, as described in another recent study of rhIL-15 alone [15], ALT-803 administration in the current study led to a 2.5-fold increase in the percentage of intracellular granzyme B-expressing cells among CD56^{bright} NK cells (range 1.8–3.3) among the 15 and 20 $\mu\text{g}/\text{kg}$ s.c. dose cohorts (N=6, data not shown). These data indicate increased cytotoxic potential amongst an NK cell subpopulation thought primarily to release cytokines and argue that treatment with ALT-803 results in NK cell activation in addition to expansion.

Overall, the total NK cell increases during Cycle 1 were more modest in the i.v. compared to the s.c. dose cohorts, but this was likely due to the lower doses of ALT-803 administered through the i.v. route. A comparison of the 6 $\mu\text{g}/\text{kg}$ i.v. to 6 $\mu\text{g}/\text{kg}$ s.c. dose cohorts reveals that the effect on expansion of total NK cells was nearly identical at 2.5 vs. 2.6 fold-increase, but for CD8+ T cells was only 1.4-fold for i.v. vs. 3.1-fold for the s.c. route. With only 3 subjects in each of these groups, it is difficult to know if these apparently different effects of administration route on CD8+ T cell expansion are meaningful, but they provide evidence that the s.c. route positively affects an important mediator of anti-tumor responses.

Our Ki-67 flow cytometric data suggest that measurement of circulating numbers of CD8+ T cells may underestimate the *in vivo* effect of ALT-803 on CD8+ T cells. During the first week after the Day 1 dose, increased expression of the activation marker HLA-DR was also detected on CD8+ T cells. It is tempting to speculate that these activated CD8+ T cells, induced by ALT-803, may be leaving the circulation to migrate to tumor sites. While the greatest NK cell expansion and CD8+ T cell proliferation and activation appeared to occur in the 15 $\mu\text{g}/\text{kg}$ dose cohort, these findings were substantially skewed by the results from one individual with remarkable immune responses. Thus, given the induction of promising levels of NK and CD8+ T cell activation and expansion, and the lack of a maximum tolerated dose or significantly increased morbidity at the highest dose, we selected 20 $\mu\text{g}/\text{kg}$ weekly of ALT-803 for future studies.

The results from this trial, obtained in parallel with those from a separate trial featuring similar ALT-803 dosing and schedule in patients with relapsed hematopoietic malignancies after allogeneic transplant [9], and a slightly later trial of ALT-803 in combination with anti-PD-1 blockade [12], are encouraging for the future of this agent. In all settings, s.c. ALT-803 was found to be safe and well-tolerated, with self-limited injection site reactions as the main adverse event. Evidence of lymphocytic infiltrates within the injection site reactions were

documented here and in the post-transplant study [9]. While immunogenicity was not observed here or post-transplant, some anti-drug antibodies were detected when ALT-803 was combined with checkpoint blockade; notably, these did not affect efficacy, safety or biologic activity. All trials demonstrated robust NK cell expansion and lesser CD8+ T cell expansion.

During the conduct of this trial, several other studies were initiated based upon our interim results that permitted the selection of a dose, route and schedule for combination. Current trials in humans include ALT-803 combined with anti-CD20 antibody in B cell malignancies, intravesical use with BCG for superficial bladder cancer, intraperitoneal and s.c. dosing in ovarian cancer, and expansion of the study with PD-1 blockade in lung cancer [13].

In conclusion, IL-15 has been considered a powerful cytokine with remarkable potential for the immunotherapy of cancer. The ALT-803 (IL-15N72D:IL-15Ra.Su/IgG1 Fc) complex, engineered to deliver stimulatory signals to NK and CD8+ T cells in order to expand memory and enhance cytotoxicity, was found to do so here in patients with advanced solid tumors. Results from this trial led to the selection of 20 µg/kg/dose s.c weekly as the optimal dose and route of delivery for this agent, and served as the foundation for the development of several other studies, now ongoing, that will reveal the true potential of this agent. Currently available data suggests ALT-803's most promising application to be complementary to other immunomodulators, including antitumor antibodies, immune checkpoint blockade, vaccine strategies, and adoptive T and NK cell therapies for a variety of solid tumors and hematologic malignancies [16].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TRANSLATIONAL RELEVANCE

ALT-803 (IL-15N72D:IL-15R α .Su/IgG1 Fc complex, Altor BioScience) was engineered to deliver stimulatory signals to NK and CD8+ T cells to enhance anti-tumor responses. The safety, tolerability, pharmacokinetics, and immunologic effects of intravenous and subcutaneous ALT-803 were evaluated in this first-in-human Phase I trial of patients with advanced solid tumors. These results led to the selection of an optimal dose and route of delivery for this agent that is safe, well-tolerated, and induces NK and CD8+ T cell proliferation and activation; this dose and route are now being utilized in ongoing clinical trials. Future studies will reveal the true potential of this agent in combination with other immunotherapies for a variety of cancers.

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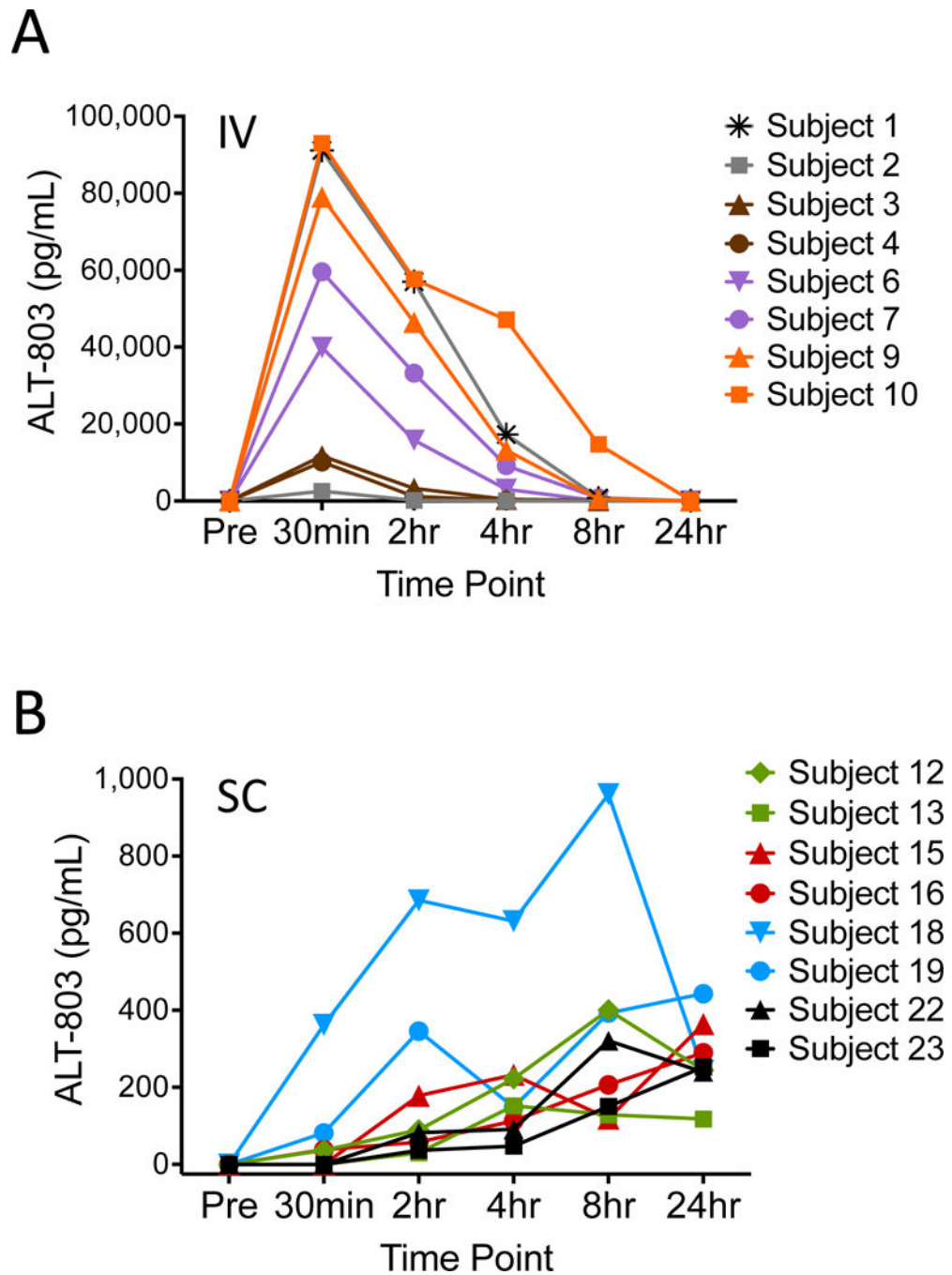


Figure 1. ALT-803 pharmacokinetics

ALT-803 levels were measured in serum samples obtained prior to (Pre) and 30 minutes, and 2, 4, 8 and 24 hours after the first dose of ALT-803 given intravenously (A), or subcutaneously (B) at Altor BioScience using a qualified ELISA as described in Materials and Methods. Individual subject levels are shown. Subjects are grouped according to dose cohort by color. ALT-803 levels in subjects receiving i.v. doses shown in panel (A) were 0.3 $\mu\text{g}/\text{kg}$ (black), 0.5 $\mu\text{g}/\text{kg}$ (gray), 1 $\mu\text{g}/\text{kg}$ (brown), 3 $\mu\text{g}/\text{kg}$ (lavender), and 6 $\mu\text{g}/\text{kg}$ (orange). Subjects receiving s.c. doses shown in panel (B) were 6 $\mu\text{g}/\text{kg}$ (green), 10 $\mu\text{g}/\text{kg}$ (red), 15

µg/kg (blue), and 20 µg/kg (black). Only two subjects per dose cohort had samples tested in these analyses, as prescribed by the study protocol.

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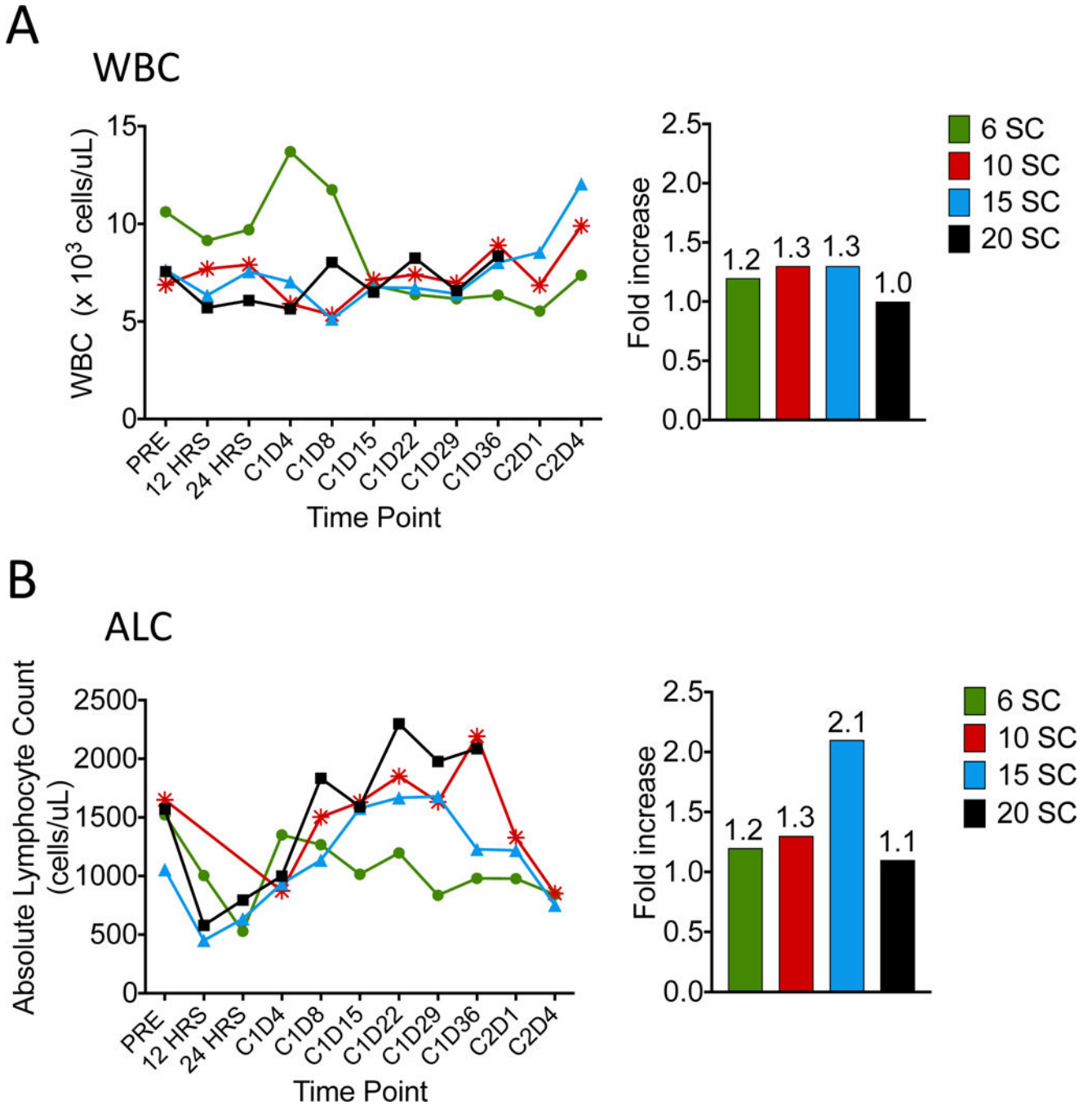


Figure 2. Effect of ALT-803 treatment on white blood cell and absolute lymphocyte counts
Circulating white blood cell (WBC x 1000 cells/ μ L, A) and absolute lymphocyte counts (ALC, cells/ μ L, B), before and during s.c. ALT-803 treatment on Days 1, 8, 15 and 22 of each 6-week cycle. The mean WBC or ALC values from subjects enrolled in each dose cohort [6 μ g/kg (green, N=3), 10 μ g/kg (red, N=3), 15 μ g/kg (blue, N=4), and 20 μ g/kg (black, N=3)] are shown in the left-hand panels. The mean maximal fold increases for each dose cohort are shown in the right-hand panels, where columns represent the mean of the

maximal fold increase for each subject during treatment compared to baseline (Day 1). The results shown were limited to data through Cycle 2 Day 4 to limit complexity.

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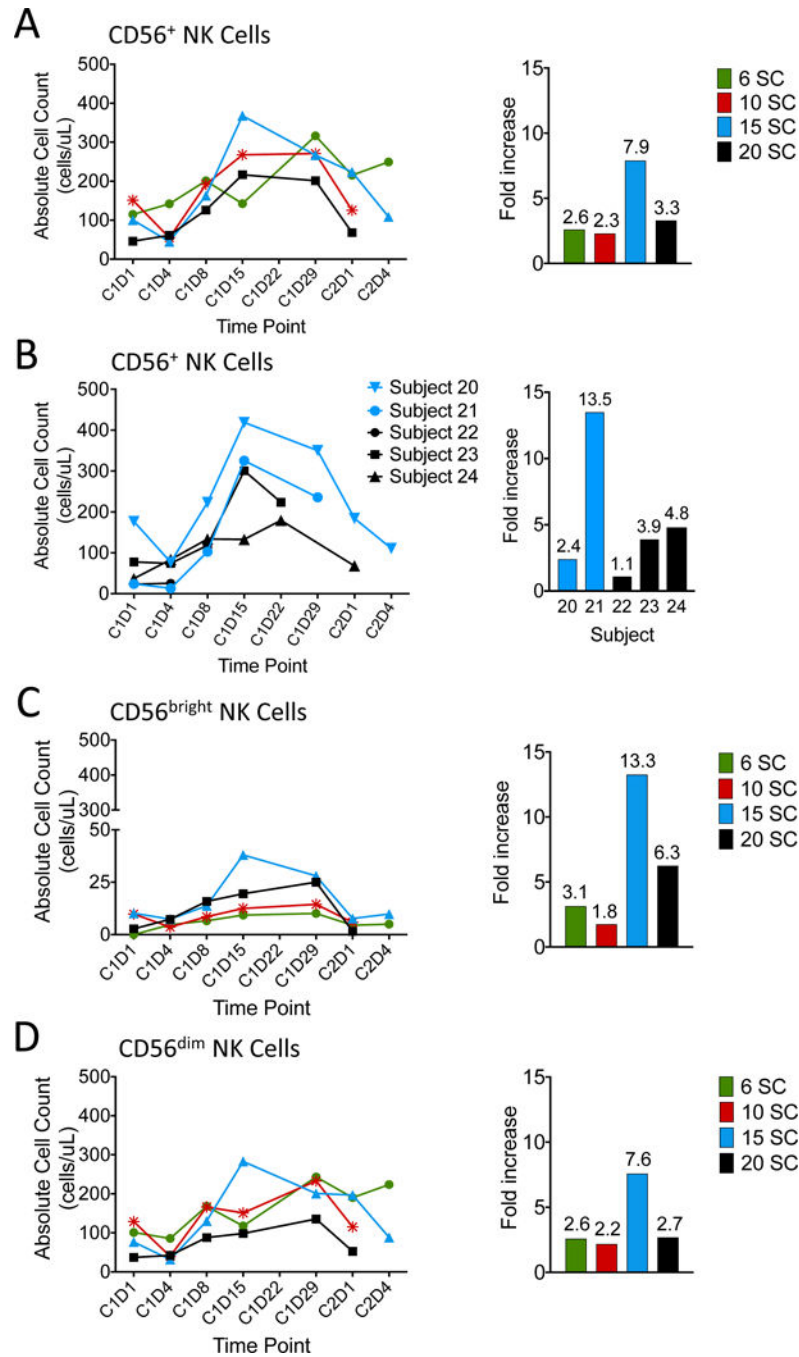


Figure 3. Circulating NK cell subset expansion during ALT-803 treatment

Data shown are from subjects who received s.c. ALT-803 on Days 1, 8, 15 and 22 of each 6-week cycle. Absolute cell frequencies are shown as means grouped by dose cohort in left panels: 6 $\mu\text{g}/\text{kg}$ (green, N=3), 10 $\mu\text{g}/\text{kg}$ (red, N=3), 15 $\mu\text{g}/\text{kg}$ (blue, N=4), and 20 $\mu\text{g}/\text{kg}$ (black, N=3). Circulating total CD56⁺ NK cells (A), and subsets CD56^{bright} (C) and CD56^{dim} (D) NK cell means for each dose cohort are represented by a single line/symbol. Total CD56⁺ NK cell numbers for individual subjects who received 15 $\mu\text{g}/\text{kg}$ (blue lines/symbols) and 20 $\mu\text{g}/\text{kg}$ (black lines/symbols) doses of ALT-803 are also shown (B),

illustrating the inter-individual variability among patients at the same dose level. In the right panels, mean maximal fold-increases during treatment compared to baseline (Day 1) for each dose cohort are indicated. The results shown were limited to data through Cycle 2 Day 4 to limit complexity, since maximal increases in NK cell counts occurred prior to that time point.

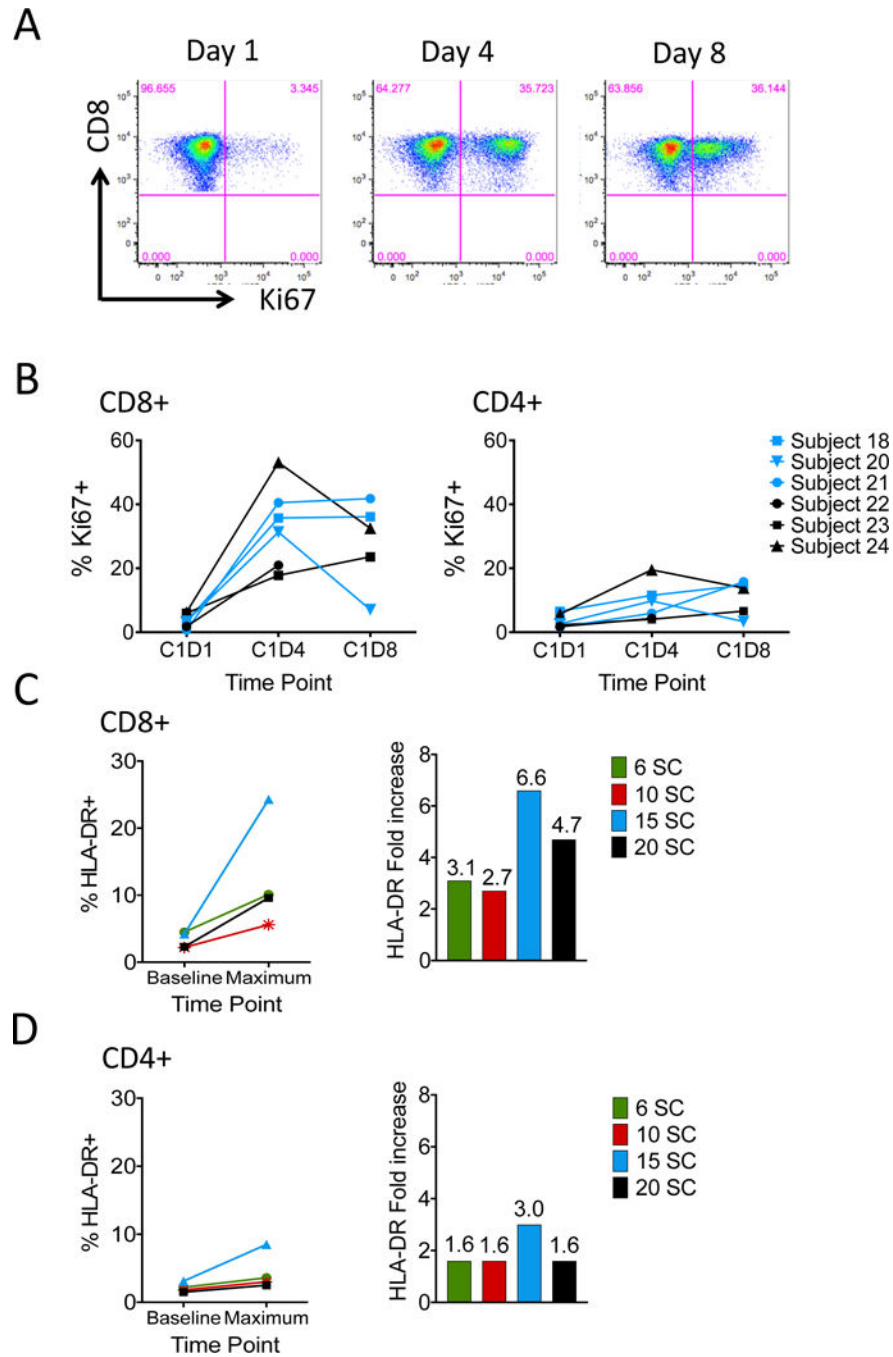


Figure 4. Effect of subcutaneous ALT-803 on circulating T cells

Representative plots of Ki-67 expression among CD3+CD8+ T cells at Days 1 (pre-treatment), 4 and 8 were generated as described in Materials and Methods (A). Results from individual subjects in the 15 µg/kg (blue) and 20 µg/kg (black) dose cohorts are shown as the percentage of Ki-67+ cells among CD8+ (left panel) or CD4+ (right panel) T cells at Days 1, 4 and 8 (B). Mean % HLA-DR expression among T cells at Day 1 (pre-treatment) and maximal time points during treatment are shown, grouped by dose cohort in left panels: 6 µg/kg s.c. (green, N=3), 10 µg/kg s.c. (red, N=3), 15 µg/kg s.c. (blue, N=4), and 20 µg/kg

s.c. (black, N=3) (C,D). Mean maximal fold-increases during treatment compared to Day 1 (pre-treatment) for each dose cohort are indicated in right panels.

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Table 1.

Subject Demographics

Subject	Age (yrs)	Gender	Cancer Type	Dose ($\mu\text{g}/\text{kg}$)	Route	Cycles Completed
1	49	F	Melanoma	0.3	IV	2
2	27	F	Melanoma	0.5	IV	2
3	67	M	Melanoma	1	IV	1
4	70	M	Melanoma	1	IV	1
5	57	M	Melanoma	1	IV	3
6	70	F	NSCLC	3	IV	1
7	50	F	Renal	3	IV	3
8	56	M	Melanoma	3	IV	1
9	62	M	Renal	6	IV	<1 *
10	69	M	Melanoma	6	IV	1
11	78	F	NSCLC	6	IV	1
12	65	M	Renal	6	SC	2
13	58	M	Melanoma	6	SC	2
14	55	F	SCCHN	6	SC	<1 **
15	61	M	Melanoma	10	SC	2
16	55	M	Renal	10	SC	1
17	61	F	Renal	10	SC	<1 ***
18	78	F	NSCLC	15	SC	2
19	57	F	NSCLC	15	SC	2 [^]
20	59	M	Renal	15	SC	1
21	59	F	SCCHN	15	SC	1
22	43	F	NSCLC	20	SC	<1 ****
23	64	M	SCCHN	20	SC	1
24	59	M	NSCLC	20	SC	1

* Subject 9 received 3 doses, experienced grade 3 dyspnea, then discontinued for disease progression.

** Subject 14 received 1 dose, experienced grade 3 hypokalemia, then withdrew consent.

*** Subject 17 received 3 doses (on Days 1, 8, and 22; held on Day 15), then discontinued for disease progression.

**** Subject 22 received 1 dose, experienced grade 3 generalized muscle weakness, then discontinued for disease progression.

[^] Subject 19 received 1 dose at 15 $\mu\text{g}/\text{kg}$ s.c.; then decreased to 10 $\mu\text{g}/\text{kg}$ due to transient grade 4 neutropenia.

Table 2.

Adverse Events in Subjects Treated with Intravenous or Subcutaneous ALT-803

Intravenous ALT-803			Highest AE Grade/Dose Cohort			
Adverse Event	# IV Subjects Affected, Total (%) N=11	# IV Subjects Affected, 3 & 6 mcg/kg N=6	0.3–0.5 mcg/kg N=2	1 mcg/kg N=3	3 mcg/kg N=3	6 mcg/kg N=3
Fatigue	6 (55)	3 (50)	1	2	1	1
Nausea	6 (55)	3 (50)	2	1	1	1
Vomiting	4 (36)	2 (33)	1	2	1	-
Chills	4 (36)	1 (17)	1	1	-	2
Fever	3 (27)	2 (33)	1	-	1	2
Subcutaneous ALT-803			Highest AE Grade/Dose Cohort			
Adverse Event	# SC Subjects Affected, Total (%) N=13	# SC Subjects Affected, 15 & 20 mcg/kg N=7	6 mcg/kg N=3	10 mcg/kg N=3	15 mcg/kg N=4	20 mcg/kg N=3
Injection site reaction	11 (85)	7 (100)	2	2	2	1
Fatigue	7 (54)	4 (57)	-	2	2	2
Hypoalbuminemia	6 (46)	4 (57)	2	2	2	2
Anemia	5 (38)	4 (57)	-	2	3	2
Fever	5 (38)	2 (29)	1	2	2	2
Lymphocyte count decreased	4 (31)	3 (43)	-	3	4	2
Limb Edema	3 (23)	3 (43)	-	-	1	1
Anorexia	3 (23)	2 (29)	-	2	2	2
Arthralgia	3 (23)	2 (29)	-	2	1	-
Vomiting	3 (23)	2 (29)	-	2	1	-

Adverse events occurring in 3 or more subjects are included.