Clinical Cancer Research

Phase I Trial of ALT-803, A Novel Recombinant IL15 Complex, in Patients with Advanced Solid Tumors

Kim Margolin[1], Chihiro Morishima[2,3], Vamsidhar Velcheti[4], Jeffrey S. Miller[5], Sylvia M. Lee[6], Ann W. Silk[7], Shernan G. Holtan[5], Andreanne M. Lacroix[3], Steven P. Fling[3], Judith C. Kaiser[3], Jack O. Egan[8], Monica Jones[8], Peter R. Rhode[8], Amy D. Rock[8], Martin A. Cheever[3], Hing C. Wong[8], and Marc S. Ernstoff[9]

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/24/22/5552/2048673/5552.pdf by guest on 01 March 2024

Abstract

Purpose: IL15 induces the activation and proliferation of natural killer (NK) and memory CD8$^+$ T cells and has preclinical antitumor activity. Given the superior activity and favorable kinetics of ALT-803 (IL15N72D:IL15RαSu/IgG1 Fc complex) over recombinant human IL15 (rhIL15) in animal models, we performed this first-in-human phase I trial of ALT-803 in patients with advanced solid tumors.

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

Results: Twenty-four patients were enrolled; 11 received intravenous and 13 received subcutaneous ALT-803. Of these patients, nine had melanoma, six renal, three head and neck, and six 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 intravenous administration, whereas painful injection site wheal was reported most commonly with subcutaneous 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 patients with advanced cancer. These data, together with compelling evidence of synergy in preclinical and clinical studies, provide the rationale for combining ALT-803 with other anticancer agents. *Clin Cancer Res; 24(22); 5552–61. ©2018 AACR.*

Introduction

IL15 is one of six known common γ chain (γc) cytokines that activate T cells and natural killer (NK) cells. The IL15 receptor shares both the β and γ chains with the IL2 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. Although the IL2α receptor chain is associated with shared β and γ chains on target lymphocytes, the membrane-associated IL15 α receptor on antigen-presenting cells (APC) binds the IL15 molecule and presents it to the βγc receptors located on the surface of T and NK cells. Functional differences include expansion and maintenance of regulatory T cells (Treg) by IL2 but not IL15; activation-induced cell death stimulated by IL2 but not by IL15; maintenance of memory CD8$^+$ T cells by IL15 but not by IL2; and a major role in controlling the lymphocyte pool size, including reconstitution following lymphodepleting therapy, by IL15 but not IL2 (1).

IL15 has shown antitumor activity as a single agent and in combinations with therapeutic antibodies or immune checkpoint–blocking antibodies in animal models (2, 3). However, IL15 alone is less likely to mediate favorable immunomodulatory effects, because it is normally trans-presented to CD8$^+$ T and NK cells in a complex with the IL15α receptor by APCs. Furthermore, the unmodified recombinant IL15 molecule has a short serum half-life and, when administered intravenously, can cause inflammatory and hemodynamic toxicities similar to those of IL2 (4), which is now used rarely for cancer therapy due to its unfavorable

[1]City of Hope National Medical Center, Duarte, California. [2]University of Washington, Seattle, Washington. [3]Cancer Immunotherapy Trials Network, Fred Hutchinson Cancer Research Center, Seattle, Washington. [4]Cleveland Clinic, Cleveland, Ohio. [5]University of Minnesota, Minneapolis, Minnesota. [6]Seattle Cancer Care Alliance, Seattle, Washington. [7]Rutgers Cancer Institute of New Jersey, New Brunswick, New Jersey. [8]Altor BioScience, a Nantworks Company, Miramar, Florida. [9]Roswell Park Cancer Center, Buffalo, New York.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

K. Margolin and C. Morishima are co-first authors of this article.

Corresponding Author: Kim Margolin, City of Hope Department of Medical Oncology, 1500 E. Duarte Rd., Duarte, CA 91010. Phone: 626-218-6007; Fax: 626-218-8233; E-mail: kmargolin@coh.org

doi: 10.1158/1078-0432.CCR-18-0945

©2018 American Association for Cancer Research.

AACR

Translational Relevance

ALT-803 (IL15N72D:IL15RαSu/IgG1 Fc complex; Altor BioScience) was engineered to deliver stimulatory signals to NK and $CD8^+$ T cells to enhance antitumor 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.

therapeutic ratio. More recently, IL15 has been developed as a therapeutic immune complex in association with the IL15α receptor or with a nonneutralizing anti-IL15 antibody (5). ALT-803 is a complex containing two molecules of an optimized amino acid–substituted (N72D) IL15 "superagonist," two molecules of the IL15α receptor "sushi" domain fused to a dimeric human IgG1 Fc that confers stability and prolongs the half-life of the overall complex (IL15N72D:IL15RαSu/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 intravenous ALT-803 weekly in humans (6, 7). Later murine studies showing eightfold lower maximum serum concentration (C_{max}) with subcutaneous versus intravenous administration, suggesting a decreased likelihood of cytokine-related systemic symptoms with subcutaneous dosing, as well as evidence of comparable immunostimulatory effects (7, 8), made the outpatient subcutaneous route of administration attractive for evaluation in patients.

This 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 intravenously or subcutaneously administered ALT-803 patients with advanced solid tumors who had failed standard therapy for their malignancies. The five 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.

Patients 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 3,000/μL, absolute lymphocyte count (ALC) at least 500/μL, absolute neutrophil count (ANC) at least 1,000/μL, platelets at least 100,000/μL, serum bilirubin and creatinine within institutional normal limits, and serum hepatic transaminases <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, 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 five 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 May 2014 and July 2017. The trial was approved and monitored by the Cancer Therapy Evaluation Program (CTEP) as well as the investigational agent sponsor, Altor BioScience, 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 (DLT) 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 intravenous dose levels passed the safety assessments, three patients were then treated at each dose level using a modified Fibonacci dose escalation scheme. If a DLT occurred in one of the first three patients enrolled at a dose level, the cohort size was expanded to six patients. If ≥two of three or six patients experienced DLTs, dose escalation would be halted and the prior level considered the maximum tolerated dose (MTD). DLTs were defined, based on previous clinical studies with rhIL15, as any ≥grade 3 toxicity except for the following: grade 3 fatigue or anorexia; grade 3 hypotension; grade 3 hypocalcemia, hypokalemia, hypomagnesemia or hyponatremia, hypophosphatemia that responded to medical intervention; fever of <41°C for <12 hours; grade 3 or 4 nausea; vomiting or diarrhea <72 hours; grade 3 injection site reaction not requiring operative

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/24/22/5552/2048673/5552.pdf by guest on 01 March 2024

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. ALC >25,000/μL 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, ALC >35,000/μL was considered a DLT.

Investigational agent

ALT-803 is a soluble complex consisting of two human IL15 variant (N72D) molecules bound to a dimeric IL15R 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 intravenously or subcutaneously each week 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 two to three injections due to volume.

Clinical and investigational assessments

Complete blood counts. Complete blood count data, including total WBC 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 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 peripheral blood mononuclear cells (PBMC) using standard Ficoll–Hypaque isolation immediately upon receipt. PBMCs were cryopreserved in 10% DMSO (Sigma) and 12.5% HSA (Gemini) 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) and CD4 (RPA-T4), CD19 (HIB19), and HLA-DR (L243; all BioLegend) after overnight shipping to the CITN Central Lab, using a method adapted from Hensley and colleagues (12). Samples were treated with BD FACS 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 Ki67 was analyzed using thawed PBMCs 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). Data analysis was performed using FlowJo software (Treestar).

Pharmacokinetic 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 intravenously or subcutaneously 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 IL15-specific ELISA Kit (R&D Systems) 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 time point (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 dilution2 series (1:100–1:6,400) and considered positive for anti–ALT-803 antibodies if the average uncorrected optical density (OD) of the postdose sample was greater than twice the average uncorrected OD of the corresponding predose 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 intravenous 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 outpatient basis and based on additional preclinical data from Altor BioScience (8), the route of administration was changed to subcutaneous after initiation of this study. A total of 13 patients were then treated with subcutaneous dose levels of 6, 10, 15, and 20 μg/kg/dose (three 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

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/24/22/5552/2048673/5552.pdf by guest on 01 March 2024

Table 1. Subject demographics

Subject	Age (years)	Gender	Cancer type	Dose (μg/kg)	Route	Cycles completed
1	49	F	Melanoma	0.3	i.v.	2
2	27	F	Melanoma	0.5	i.v.	2
3	67	M	Melanoma	1	i.v.	1
4	70	M	Melanoma	1	i.v.	1
5	57	M	Melanoma	1	i.v.	3
6	70	F	NSCLC	3	i.v.	1
7	50	F	Renal	3	i.v.	3
8	56	M	Melanoma	3	i.v.	1
9	62	M	Renal	6	i.v.	<1[a]
10	69	M	Melanoma	6	i.v.	1
11	78	F	NSCLC	6	i.v.	1
12	65	M	Renal	6	s.c.	2
13	58	M	Melanoma	6	s.c.	2
14	55	F	SCCHN	6	s.c.	<1[b]
15	61	M	Melanoma	10	s.c.	2
16	55	M	Renal	10	s.c.	1
17	61	F	Renal	10	s.c.	<1[c]
18	78	F	NSCLC	15	s.c.	2
19	57	F	NSCLC	15	s.c.	2[d]
20	59	M	Renal	15	s.c.	1
21	59	F	SCCHN	15	s.c.	1
22	43	F	NSCLC	20	s.c.	<1[e]
23	64	M	SCCHN	20	s.c.	1
24	59	M	NSCLC	20	s.c.	1

Abbreviations: F, female; M, male; NSCLC, non–small cell lung cancer; SCCHN, squamous cell carcinoma of the head and neck.
[a]Subject 9 received three doses, experienced grade 3 dyspnea, and then discontinued for disease progression.
[b]Subject 14 received one dose, experienced grade 3 hypokalemia, and then withdrew consent.
[c]Subject 17 received three doses (on days 1, 8, and 22; held on day 15), and then discontinued for disease progression.
[d]Subject 19 received one dose at 15 μg/kg s.c., then decreased to 10 μg/kg due to transient grade 4 neutropenia.
[e]Subject 22 received one dose, experienced grade 3 generalized muscle weakness, and then discontinued for disease progression.

decreased dose of 10 μg/kg. Over both intravenous and subcutaneous dose cohorts, nine patients had melanoma, six renal cancer, three head and neck squamous cell cancer, and six non–small cell lung cancer.

Eleven of 24 subjects receiving intravenous or subcutaneous ALT-803 completed one 6-week cycle of therapy; seven subjects completed two cycles; two completed three cycles; and four subjects completed <1 cycle, of whom three 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 intravenous and subcutaneous routes of administration (Table 2), except for Subject 19, whose dose was reduced for grade 4 neutropenia. For simplicity, only adverse events reported for three or more subjects are shown in Table 2. The toxicity profile for the intravenous 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 intravenous dose cohorts, and was described in only two subjects in the subcutaneous dose cohorts (one 15 μg/kg subject and one 20 μg/kg subject), both grade 1. For the subcutaneous 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 a single agent or in combination with a checkpoint inhibitor (9, 13).

One patient with lung cancer (Subject 24) received one cycle of ALT-803 at the highest dose of 20 μg/kg s.c., had a normal prestudy 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 four-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, because 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.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/24/22/5552/2048673/5552.pdf by guest on 01 March 2024

Table 2. Adverse events in subjects treated with intravenous or subcutaneous ALT-803

	Intravenous ALT-803		Highest AE grade/dose cohort			
Adverse event	**No. i.v. Subjects affected, total (%) $n = 11$**	**No. i.v. Subjects affected, 3 and 6 µg/kg $n = 6$**	**0.3–0.5 µg/kg $n = 2$**	**1 µg/kg $n = 3$**	**3 µg/kg $n = 3$**	**6 µg/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	**No. s.c. Subjects affected, total (%) $n = 13$**	**No. s.c. Subjects affected, 15 and 20 µg/kg $n = 7$**	**6 µg/kg $n = 3$**	**10 µg/kg $n = 3$**	**15 µg/kg $n = 4$**	**20 µg/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	—

NOTE: Adverse events occurring in three or more subjects are included.

Pharmacokinetics, immunogenicity, and pharmacodynamics

Pharmacokinetic data were obtained predose and at 30 minutes, 2, 4, 8, and 24 hours after ALT-803 dosing, from two subjects in each dose cohort according to the study protocol (Fig. 1). The time to maximum serum concentration (T_{max}) occurred consistently at 30 minutes after intravenous administration. In contrast, subcutaneous ALT-803 exhibited a very gradual increase in serum concentration, with maximal serum levels >100-fold less than the average peak after intravenous ALT-803. In four of eight subcutaneous 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 four subjects.

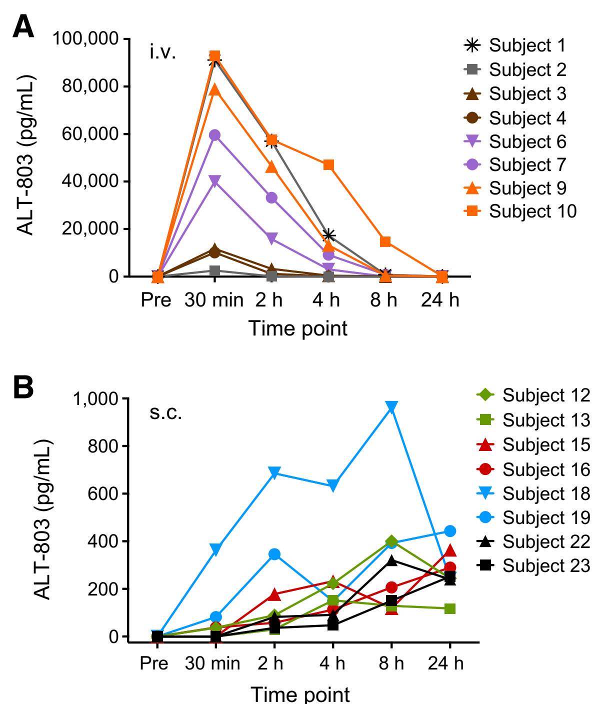

Figure 1.
ALT-803 pharmacokinetics. ALT-803 levels were measured in serum samples obtained prior to (Pre) and 30 minutes, and 2, 4, 8, and 24 hours after the first dose of ALT-803 given intravenously (**A**) or subcutaneously (**B**) at Altor BioScience using a qualified ELISA as described in Materials and Methods. Individual subject levels are shown. Subjects are grouped according to dose cohort by color. ALT-803 levels in subjects receiving intravenous doses shown in **A** were 0.3 µg/kg (black), 0.5 µg/kg (gray), 1 µg/kg (brown), 3 µg/kg (lavender), and 6 µg/kg (orange). Subjects receiving subcutaneous doses shown in **B** were 6 µg/kg (green), 10 µg/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.

Immunogenicity was tested on samples collected at baseline, prior to dosing on cycle 2 day 1 (C2D1), and at the 14D FU time point by Altor BioScience, as described. Eight of 11 and 8 of 13 subjects treated with intravenous and subcutaneous ALT-803, respectively, were tested at the 14D FU time point; and one and three 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 WBC count and calculated ALC were primary endpoints for this phase I trial, which sought to evaluate the impact of ALT-803 on the expansion of IL15-responsive lymphocytes. ALT-803 demonstrated little effect on overall WBC counts when maximal counts during cycle 1

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/24/22/5552/2048673/5552.pdf by guest on 01 March 2024

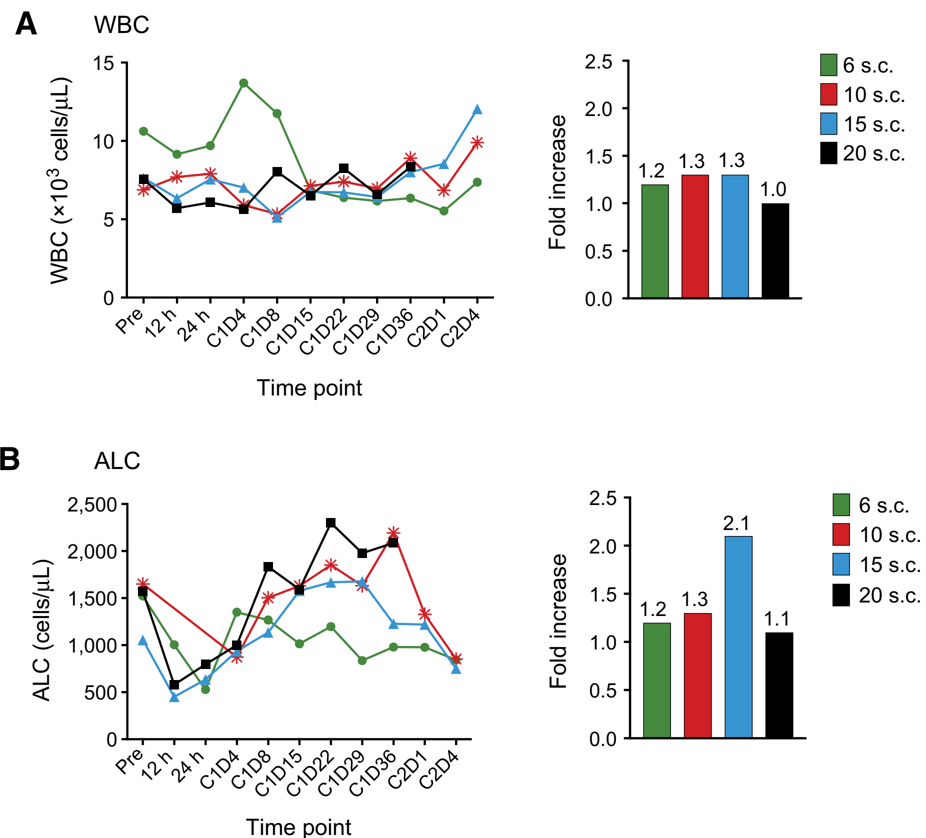

Figure 2.
Effect of ALT-803 treatment on WBC and absolute lymphocyte counts. Circulating WBCs (×1,000 cells/µL, **A**) and ALC (cells/µL, **B**), before and during subcutaneous ALT-803 treatment on days 1, 8, 15, and 22 of each 6-week cycle. The mean WBC or ALC values from subjects enrolled in each dose cohort [6 µg/kg (green, $n = 3$), 10 µg/kg (red, $n = 3$), 15 µg/kg (blue, $n = 4$), and 20 µg/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 with baseline (day 1). The results shown were limited to data through cycle 2 day 4 to limit complexity.

were compared with baseline counts (subcutaneous range 1.0–1.3-fold increase, Fig. 2A, intravenous range 1.1–1.3-fold increase; Supplementary Fig. S1A). The ALC transiently decreased after the first dose of ALT-803, reaching a nadir between 12 hours and 4 days postdose for subcutaneous dosing (Fig. 2B) and 12 to 24 hours for intravenous dosing (Supplementary Fig. S1B). Subsequently, the ALC recovered but in most cases did not rise much higher than baseline over the course of cycle 1 for the subcutaneous dose cohorts, with the exception of a maximal 2.1-fold increase occurring in the 15 µg/kg dose cohort, peaking 1 to 2 weeks after the last dose of ALT-803 during cycle 1. The mean maximal posttreatment ALC increases during cycle 1 were 1.2, 1.3, 2.1, and 1.1-fold, respectively, for the four subcutaneous 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 three cycles (the remainder of her participation in the study). For the four intravenous dose cohorts, the mean maximal posttreatment ALC increases during cycle 1 were 1.1, 1.4, 1.5, and 1.6-fold respectively for the 0.3 and 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 subcutaneous dose cohorts, as was observed for the ALC, but not for the intravenous 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 intravenous (Supplementary Fig. S2) and subcutaneous dose cohorts (Fig. 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 subcutaneous 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 subcutaneous dose cohort had useable flow cytometry data available (Fig. 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 intravenous dose cohorts, the mean maximal posttreatment 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 and 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 subcutaneous dose cohorts (3.1-, 1.8-, 13.3-, and 6.3-fold for 6, 10, 15, and 20 µg/kg, respectively) compared with the larger $CD56^{dim}$ subpopulation (2.6-, 2.2-, 7.6-, and 2.7-fold for 6, 10, 15, and 20 µg/kg, respectively; Fig. 3C and D). Similarly, for the highest intravenous dose cohort of 6 µg/kg/dose, the

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/24/22/5552/2048673/5552.pdf by guest on 01 March 2024

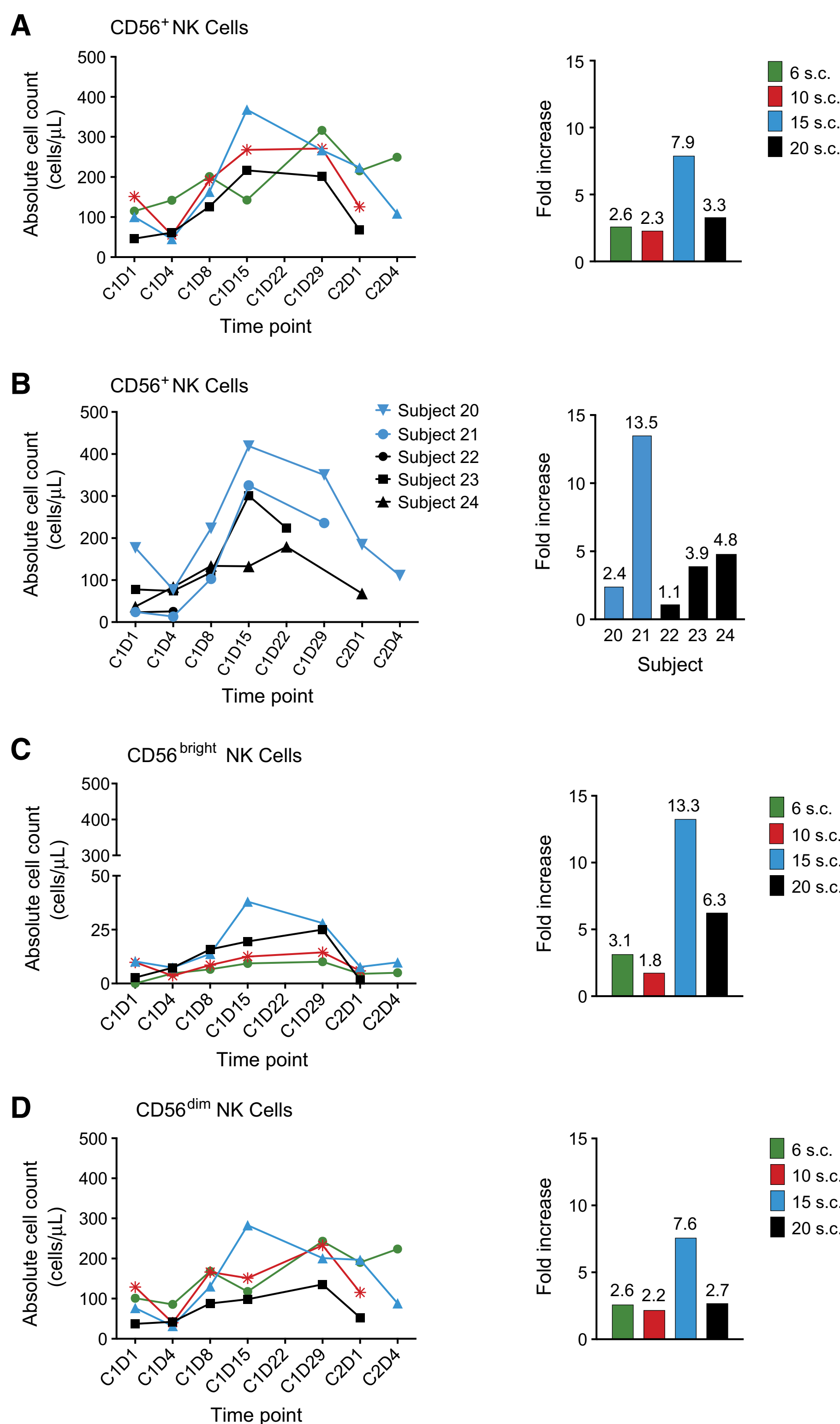

Figure 3.
Circulating NK cell subset expansion during ALT-803 treatment. Data shown are from subjects who received subcutaneous ALT-803 on days 1, 8, 15, and 22 of each 6-week cycle. Absolute cell frequencies are shown as means grouped by dose cohort on the left: 6 μg/kg (green, $n = 3$), 10 μg/kg (red, $n = 3$), 15 μg/kg (blue, $n = 4$), and 20 μg/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 μg/kg (blue lines/symbols) and 20 μg/kg (black lines/symbols) doses of ALT-803 are also shown (**B**), illustrating the interindividual variability among patients at the same dose level. On the right, mean maximal fold increases during treatment compared with baseline (day 1) for each dose cohort are indicated. The results shown were limited to data through cycle 2 day 4 to limit complexity, because maximal increases in NK cell counts occurred prior to that time point.

$CD56^{bright}$ subpopulation demonstrated a greater fold increase (5.3-fold) compared with the $CD56^{dim}$ NK cells (2.6-fold; Supplementary Fig. S2). 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 IL15 agents (14).

In contrast, overall circulating $CD4^+$ and $CD8^+$ T cell numbers (including $CD8^+$ memory T cells) did not increase substantially

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/24/22/5552/2048673/5552.pdf by guest on 01 March 2024

with ALT-803 treatment; overall means were 1.1- and 1.3-fold increases respectively, for subcutaneous, and 1.3- and 1.5-fold increases respectively, for intravenous 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 numbers was observed for the remaining 6 subjects with available data (mean 1.1-fold increase, data not shown).

Interestingly, intracellular analyses with a fluorescently labeled antibody specific for Ki67, 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 (Fig. 4A and B). Subject 21 (15 μg/kg s.c.) exhibited a remarkable response to ALT-803, with 63- and 9.8-fold increases in Ki67 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 threefold 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 subcutaneous ALT-803 dosing, a larger

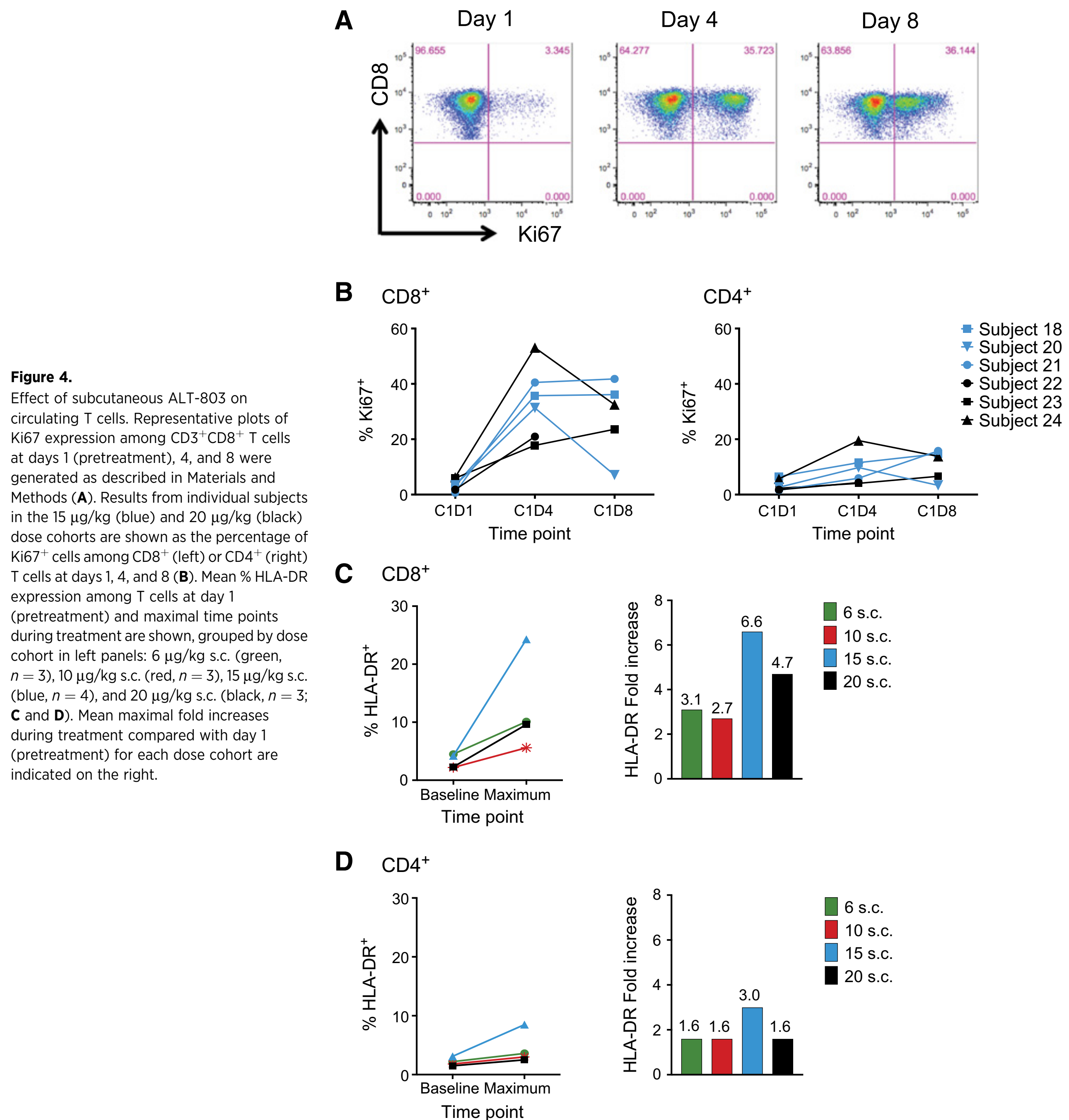

Figure 4.
Effect of subcutaneous ALT-803 on circulating T cells. Representative plots of Ki67 expression among $CD3^+CD8^+$ T cells at days 1 (pretreatment), 4, and 8 were generated as described in Materials and Methods (**A**). Results from individual subjects in the 15 μg/kg (blue) and 20 μg/kg (black) dose cohorts are shown as the percentage of $Ki67^+$ cells among $CD8^+$ (left) or $CD4^+$ (right) T cells at days 1, 4, and 8 (**B**). Mean % HLA-DR expression among T cells at day 1 (pretreatment) 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** and **D**). Mean maximal fold increases during treatment compared with day 1 (pretreatment) for each dose cohort are indicated on the right.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/24/22/5552/2048673/5552.pdf by guest on 01 March 2024

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, 10, 15, 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, 10, 15, and 20 μg/kg dose cohorts). The highest level of intracellular Ki67 expression occurred on either days 4 or 8 after the first dose of ALT-803, whereas maximal expression of HLA-DR on $CD8^+$ T cells occurred consistently on day 4 (with only one exception at day 8). Increases in PD-1–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 intravenous dose cohorts for both $CD4^+$ (range, 1.2–1.5-fold change) and $CD8^+$ (range, 1.2–2.3-fold change) T cells (Supplementary Fig. S3). Interestingly, most of these increases were maximal later during cycle 1 in the intravenous compared with the subcutaneous dose cohorts, with nine 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 intravenous or subcutaneous ALT-803. Despite local injection site reactions occurring in 85% of subcutaneously treated patients, a formally defined MTD 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 intravenous and subcutaneous ALT-803 led to increases in circulating NK cells, with the greatest fold increases occurring in the $CD56^{bright}$ NK cell subpopulation. Although circulating numbers of $CD8^+$ T cells did not increase substantially, Ki67 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 intravenous and subcutaneous dose cohorts. Intravenous administration led to maximum serum concentrations (C_{max}) >100-fold higher compared with subcutaneous, and occurred consistently early for the intravenous dose cohorts (30 minutes postdose) compared with between 8 and 24 hours for subcutaneous 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 subcutaneous 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 intravenous and subcutaneous ALT-803–treated groups, similar to results from a phase I trial of subcutaneous rhIL15 alone delivered by daily injection (14). Moreover, as described in another recent study of rhIL15 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 μg/kg subcutaneous dose cohorts (n = 6, data not shown). These data indicate increased cytotoxic potential among 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 intravenous compared with the subcutaneous dose cohorts, but this was likely due to the lower doses of ALT-803 administered through the intravenous route. A comparison of the 6 μg/kg intravenous to 6 μg/kg subcutaneous dose cohorts reveals that the effect on expansion of total NK cells was nearly identical at 2.5-fold versus 2.6-fold increase, but for $CD8^+$ T cells was only 1.4-fold for intravenous versus 3.1-fold for the subcutaneous route. With only 3 subjects in each of these groups, it is difficult to know whether these apparently different effects of administration route on $CD8^+$ T-cell expansion are meaningful, but they provide evidence that the subcutaneous route positively affects an important mediator of antitumor responses.

Our Ki67 flow cytometric data suggest that measurement of circulating numbers of $CD8^+$ T cells may understimate 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. Although the greatest NK cell expansion and $CD8^+$ T-cell proliferation and activation appeared to occur in the 15 μg/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 an MTD or significantly increased morbidity at the highest dose, we selected 20 μg/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, subcutaneous 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 posttransplant study (9). Although immunogenicity was not observed here or post-transplant, some antidrug 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 subcutaneous dosing in ovarian cancer, and expansion of the study with PD-1 blockade in lung cancer (13).

In conclusion, IL15 has been considered a powerful cytokine with remarkable potential for the immunotherapy of cancer. The ALT-803 (IL15N72D:IL15RαSu/IgG1 Fc) complex,

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/24/22/5552/2048673/5552.pdf by guest on 01 March 2024

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 suggest 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).

Disclosure of Potential Conflicts of Interest

K. Margolin is a consultant/advisory board member for Amgen, ImaginAb, Iovance, and Nektar. V. Velcheti is a consultant/advisory board member for Astrazenca, Bristol-Myers Squibb, Genentech, and Merck. S.G. Holtan is a consultant/advisory board member for Incyte. J.O. Egan, M. Jones, P.R. Rhode, and A.D. Rock hold ownership interest (including patents) in Altor BioScience. M.S. Ernstoff is a consultant/advisory board member for Bristol-Myers Squibb, EMD Serono, and Iovance. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: K. Margolin, V. Velcheti, J.S. Miller, A.M. Lacroix, M. Jones, P.R. Rhode, M.A. Cheever, H.C. Wong, M.S. Ernstoff
Development of methodology: K. Margolin, C. Morishima, V. Velcheti, A.M. Lacroix, J.O. Egan,
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. Margolin, C. Morishima, V. Velcheti, S.M. Lee, A.W. Silk, S.G. Holtan, S.P. Fling, J.C. Kaiser, J.O. Egan, M.S. Ernstoff
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K. Margolin, C. Morishima, V. Velcheti, A.W. Silk, S.G. Holtan, S.P. Fling, J.O. Egan, A.D. Rock, M.A. Cheever, H.C. Wong, M.S. Ernstoff
Writing, review, and/or revision of the manuscript: K. Margolin, C. Morishima, V. Velcheti, J.S. Miller, S.M. Lee, A.W. Silk, S.G. Holtan, A.M. Lacroix, S.P. Fling, J.O. Egan, M. Jones, P.R. Rhode, A.D. Rock, M.A. Cheever, H.C. Wong, M.S. Ernstoff
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K. Margolin, C. Morishima, A.M. Lacroix, S.P. Fling, J.C. Kaiser, M.A. Cheever, M.S. Ernstoff
Study supervision: K. Margolin, A.M. Lacroix, S.P. Fling, J.C. Kaiser, M.A. Cheever
Other (assisted in the development of all aspects of the study including fiscal, operational components): J.C. Kaiser

Acknowledgments

The authors would like to thank the study participants and acknowledge the contributions of the following individuals to this work: Minjun Apodaca, ASCP, and Leonard D'Amico, PhD, and other members of the CITN Central Laboratory for their technical contributions; Stephen C. De Rosa MD and Tiffany Hensley-McBain of the HIV Vaccine Trials Network for their help in establishing the flow cytometric methods; the Fred Hutchinson Cancer Research Center Flow Cytometry Facility for their support in conducting testing; and Angela Riggins and Michael Watling for their help in preparation of the manuscript figures. This work was supported by Cancer Immunotherapy Trials Network (CITN): NIH 1U01 CA154967-01 (ClinicalTrials.gov NCT01727076); Melanoma Research Alliance Academic Industry Award, Altor BioScience.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received March 30, 2018; revised June 2, 2018; accepted July 13, 2018; published first July 25, 2018.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/24/22/5552/2048673/5552.pdf by guest on 01 March 2024

References

1. Waldmann TA. The shared and contrasting roles of IL2 and IL15 in the life and death of normal and neoplastic lymphocytes: implications for cancer therapy. Cancer Immunol Res 2015;3:219–27.
2. Mathios D, Park CK, Marcus WD, Alter S, Rhode PR, Jeng EK, et al. Therapeutic administration of IL-15 superagonist complex ALT-803 leads to long-term survival and durable antitumor immune response in a murine glioblastoma model. Int J Cancer 2016;138:187–94.
3. Xu W, Jones M, Liu B, Zhu X, Johnson CB, Edwards AC, et al. Efficacy and mechanism-of-action of a novel superagonist interleukin-15: interleukin-15 receptor alphaSu/Fc fusion complex in syngeneic murine models of multiple myeloma. Cancer Res 2013;73:3075–86.
4. Conlon KC, Lugli E, Welles HC, Rosenberg SA, Fojo AT, Morris JC, et al. Redistribution, hyperproliferation, activation of natural killer cells and CD8 T cells, and cytokine production during first-in-human clinical trial of recombinant human interleukin-15 in patients with cancer. J Clin Oncol 2015;33:74–82.
5. Rautela J, Huntington ND. IL-15 signaling in NK cell cancer immunotherapy. Curr Opin Immunol 2017;44:1–6.
6. Kim PS, Kwilas AR, Xu W, Alter S, Jeng EK, Wong HC, et al. IL-15 superagonist/IL-15RalphaSushi-Fc fusion complex (IL-15SA/IL-15RalphaSu-Fc; ALT-803) markedly enhances specific subpopulations of NK and memory CD8+ T cells, and mediates potent anti-tumor activity against murine breast and colon carcinomas. Oncotarget 2016;7:16130–45.
7. Rhode PR, Egan JO, Xu W, Hong H, Webb GM, Chen X, et al. Comparison of the superagonist complex, ALT-803, to IL15 as cancer immunotherapeutics in animal models. Cancer Immunol Res 2016;4:49–60.
8. Liu B, Jones M, Kong L, Noel T, Jeng EK, Shi S, et al. Evaluation of the biological activities of the IL-15 superagonist complex, ALT-803, following intravenous versus subcutaneous administration in murine models. Cytokine 2018;107:105–12.
9. Romee R, Cooley S, Berrien-Elliott MM, Westervelt P, Verneris MR, Wagner JE, et al. First-in-human phase 1 clinical study of the IL-15 superagonist complex ALT-803 to treat relapse after transplantation. Blood 2018;131:2515–27.
10. Han KP, Zhu X, Liu B, Jeng E, Kong L, Yovandich JL, et al. IL-15:IL-15 receptor alpha superagonist complex: high-level co-expression in recombinant mammalian cells, purification and characterization. Cytokine 2011;56:804–10.
11. Zhu X, Marcus WD, Xu W, Lee HI, Han K, Egan JO, et al. Novel human interleukin-15 agonists. J Immunol 2009;183:3598–607.
12. Hensley TR, Easter AB, Gerdts SE, De Rosa SC, Heit A, McElrath MJ, et al. Enumeration of major peripheral blood leukocyte populations for multicenter clinical trials using a whole blood phenotyping assay. J Vis Exp 2012;16:e4302.
13. Wrangle JM, Velcheti V, Patel MR, Garrett-Mayer E, Hill EG, Ravenel JG, 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;19:694–704.
14. Miller JS, Morishima C, McNeel DG, Patel MR, Kohrt HE, Thompson JA, et al. A first-in-human phase 1 study of subcutaneous outpatient recombinant human IL-15 (rhIL-15) in adults with advanced solid tumors. Clin Cancer Res 2018;24:1525–35.
15. Dubois S, Conlon KC, Muller JR, Hsu-Albert J, Beltran N, Bryant BR, et al. IL15 infusion of cancer patients expands the subpopulation of cytotoxic CD56(bright) NK cells and increases NK-cell cytokine release capabilities. Cancer Immunol Res 2017;5:929–38.
16. Cheever MA. Twelve immunotherapy drugs that could cure cancers. Immunol Rev 2008;222:357–68.