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Evaluation of the biological activities of the IL-15 superagonist complex, ALT-803, following intravenous versus subcutaneous administration in murine models

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ABSTRACT

ALT-803 is a fusion protein complex consisting of an interleukin (IL)-15 superagonist and a dimeric IL-15 receptor alpha sushi domain IgG1 Fc fusion protein. When administered to mice, ALT-803 is capable of inducing natural killer (NK) and $CD8^+$ T cell proliferation and activation, and effectively promoting potent anti-tumor responses. Currently, ALT-803 is in clinical trials for treatment of various solid tumors and hematological malignancies. In the initial phase of these clinical studies, intravenous (iv) injection was used according to the route used in pre-clinical efficacy studies. In order to evaluate the possible advantage of subcutaneous (sc) injection versus iv injection, this study compared the biological activity of the two treatment regimens of ALT-803 in pre-clinical *in vivo* models. The pharmacokinetics, immune stimulation, and anti-tumor efficacy of iv and sc injection routes of ALT-803 in C57BL/6 mice were compared. The half-life of ALT-803 was 7.5 h for iv versus 7.7 h for sc with the maximal detected serum concentration of ALT-803 to be 3926 ng/ml at 0.5 h time-point following iv injection versus 495 ng/ml at 16 h post sc injection. Biodistribution studies indicated that sc ALT-803, similarly to iv ALT-803 as previously reported, has a greater tissue distribution and longer residence time in lymphoid tissues compared to recombinant IL-15. Notably, ALT-803 when administered either iv or sc induced comparable proliferation and activation of $CD8^+$ T and NK cells and resulted in similar reductions of tumor burden. A toxicity study of mice receiving multiple injections of ALT-803 for 4 weeks by iv or sc routes revealed equivalent immune-related changes. The gradual absorbance into the blood stream and lower maximal blood levels of ALT-803 in sc-injected mice, along with similar anti-tumor efficacy support the administration of ALT-803 by sc injection in patients with various malignancies and infectious diseases.

1. Introduction

Cytokines have a pivotal role in the regulation of the immune system and have been approved in therapeutic applications to treat cancer. The common γ-chain cytokine interleukin (IL)-15, is a growth factor for activation and proliferation of natural killer (NK) cells and $CD8^+$ T cells [1,2]. In contrast to other common γ-chain cytokines such as IL-2, IL-15 is not associated with activation induced cell death (AICD) and does not induce capillary leak syndrome in mice and non-human primates [3,4]. IL-15 has a distinct role in prolonged maintenance of memory T-cells against invading pathogens, supporting development and activation of NK cells, and is a desirable immunotherapy candidate against cancer [4–8]. We developed an IL-15 superagonist complex, ALT-803, to circumvent the shortcomings of recombinant IL-15 and advance an IL-15-based immunotherapy into clinical evaluation for cancer and infectious diseases. As previously reported, ALT-803 is a fusion protein complex consisting of an IL-15N72D superagonist and a dimeric IL-15 receptor alpha sushi domain IgG1 Fc fusion protein (IL-15RαSu/Fc) [9,10]. ALT-803 was shown to exhibit superior immunostimulatory activity, prolonged serum half-life, and more potent anti-tumor activity compared to recombinant IL-15 in various mouse models [11,12].

Abbreviations: IL-15N72D, human IL-15 aa 72N to D variant; IL-15RαSu, human IL-15 receptor alpha sushi domain

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Clinical trials evaluating ALT-803 for treatment of solid tumors and hematologic malignancies are underway. In initial clinical trials, intravenous (iv) administration of ALT-803 showed a dose-dependent immune cell activation, which correlated with increased serum IFNγ and IL-6 levels and constitutional side effects [13]. Thus, we hypothesized that subcutaneous (sc) administration of ALT-803 may lessen the constitutional adverse events (AEs) by lowering peak serum level of ALT-803. In order to demonstrate the possible advantage of sc injection, we compared the immunologic effects and anti-tumor activity of ALT-803 using the different dosing routes in pre-clinical models. The two treatment regimens of ALT-803 were evaluated and pharmacokinetics, immune cell activity, anti-tumor activity, and biodistribution were assessed in pre-clinical *in vivo* studies. The results reported herein strongly support a treatment regimen of sc administration of ALT-803 in clinical applications.

2. Materials and methods

2.1. Mice and cell lines

Six- to eight-week old female C57BL/6NHsd mice (B6) were purchased from Envigo Corporation (Indianapolis, IN) and were housed in the animal facility at Altor BioScience (Miramar, FL). All animal studies were performed according to NIH animal care guidelines under Institutional Animal Care and Use Committee approved protocols.

The murine 5T33 multiple myeloma cell line [14] was kindly provided by Dr. Ulrich van Andrian (Harvard Medical School, Cambridge, MA). Cells were routinely cultured in RPMI-1640 (Invitrogen, Grand Island, NY) supplemented with 10% fetal calf serum (HyClone, Waltham, MA) at 37 °C with 5% CO_2.

2.2. Pharmacokinetic analysis

ALT-803 (IL-15N72D:IL-15RαSu/Fc) was generated as previously described [10]. ALT-803 (0.2 mg/kg) was administered to B6 mice (6 weeks old) by iv (tail vein) or sc (right flank) injections. A dose equivalent volume of PBS was injected into a group of B6 mice as vehicle control. Serum was prepared from blood from the tail vein at varying time-points. ALT-803 serum levels were assessed by ELISA (anti-IL-15 antibody (Ab) capture and HRP-conjugated donkey anti-human IgG Ab), which detects the intact ALT-803 complex of IL-15N72D:IL-15RαSu/Fc fusion protein [10]. The pharmacokinetics of ALT-803 were analyzed with PK Solution 2.0 software (Summit Research Services, Montrose, CO).

2.3. Immune cell activation and proliferation analysis

B6 mice were injected iv or sc with ALT-803 or PBS as described above. Mice were humanely sacrificed and spleens were harvested at 0, 16, 24, 48, and 72 h following injection. For splenic immune cell phenotyping, splenocytes were isolated and stained with the fluorochrome-conjugated monoclonal antibodies (mAbs) against mouse CD4, CD8, NK1.1, NKp46, KLRG1, CD25, and intracellular Foxp3 and granzyme B molecules (Biolegend, San Diego, CA). The stained cells were analyzed on a FACSverse with FACSuite software (BD Biosciences, San Jose, CA). Serum cytokine levels of ALT-803-treated mice were also assessed using a Cytometric Bead Array, Mouse Inflammation Kit (BD Biosciences, San Jose, CA) according to manufacturer's instructions. The data were analyzed using Flow Cytometric Analysis Program (FCAP) Array Software (BD Biosciences, San Jose, CA).

2.4. ALT-803 tissue biodistribution studies

B6 mice were injected iv or sc with 3–7 MBq of ^{64}Cu-labeled ALT-803 [12,15]. Static PET scans were performed on anesthetized animals at various time-points after injection using an Inveon microPET/microCT hybrid scanner (Siemens). Data acquisition, image reconstruction, and region-of-interest analysis to calculate the percentage injected dose per gram of tissue (%ID/g) for major organs were conducted as previously described [12,15].

2.5. Tumor model

Six-week old B6 mice were injected iv with 1×10^7 5T33 myeloma cells on day 0. On study days (SD) 10 and 14, ALT-803 (0.2 mg/kg) or PBS were administered into B6 mice by iv or sc injection. On SD17, mice were humanely sacrificed and spleen and bone marrow (BM) cells from hind leg femurs were harvested. The levels of BM myeloma cells were assessed by intracellular staining with FITC-conjugated anti-mouse IgG2b which is expressed on 5T33 tumor cells. To assess the effect of ALT-803 on immune cell stimulation in 5T33 tumor-bearing mice, NK and $CD8^+$ T cells were also evaluated. BM cells and splenocytes of tumor-bearing mice were stained with fluorochrome-conjugated mAbs against mouse CD8, KLRG1, NK1.1 or NKp46. The stained cells were analyzed as described above.

2.6. Toxicity of repeated sc injections of ALT-803

Eight-week old female B6 mice (5 mice/group) were injected sc with a high dose of ALT-803 (1.0 mg/kg) or a PBS once a week for 4 weeks (SD1, 8, 15, and 22). Cage side observations including mortality, morbidity, and signs of study drug toxicity were conducted at least three times per week. Animals were weighed prior to each dosing and on the day of sacrifice (SD26). Blood samples were collected into heparinized tubes on SD26, 4 days after administration of the final dose of ALT-803. Mice were sacrificed on SD26, necropsied, organs (spleen, peripheral lymph nodes, liver, lungs, heart, thymus, kidneys) were removed for weight determination. Hematologic and chemical analysis of the serum was performed by the Comparative Pathology Laboratory/Department of Pathology at the University of Miami, Miller School of Medicine, Miami, FL.

3. Results and discussion

3.1. Pharmacokinetics evaluation of ALT-803 following iv or sc administration

We have previously reported that in ICR (CD-1) mice receiving 1.0 mg/kg ALT-803 by iv injection, the estimated serum half-life values of ALT-803 using anti-IL-15 Ab-based or anti-human IgG Fc Ab-based ELISAs were about 25 h and 18 h, respectively [10]. To compare the pharmacokinetics of ALT-803 following iv or sc administration, groups of C57BL/6 mice (3 mice/time-point) were injected iv or sc with 0.2 mg/kg of ALT-803. Serum levels of ALT-803 were assessed at varying time-points by ELISA (anti-IL-15 Ab capture and HRP-conjugated donkey anti-human IgG Ab detection), which detected the intact complex of ALT-803. The pharmacokinetic profile of ALT-803 in these mice was analyzed using PK Solution 2.0 software and serum concentrations of ALT-803 are shown in Fig. 1. The estimated elimination half-life values of ALT-803 following iv or sc administration was 7.50 h and 7.71 h, respectively. The maximal detected serum concentrations (Cmax) of ALT-803 were 3926 ng/ml at 0.5 h time-point following iv injection and 495 ng/ml at 16 h time-point following sc injection.

This finding indicates that the serum half-life of ALT-803 following sc administration is comparable with half-life following iv administration. Due to the slower absorbance time of sc injection, we used the Cmax of ALT-803 to determine the blood concentration of ALT-803 achieved by the different routes of administration. Based on these concentrations, the Cmax of ALT-803 by iv administration was 7-fold higher than that by sc administration. Since the higher serum levels of ALT-803 may induce more frequent and severe systematic side effects related to inflammatory cytokines [13], this finding suggests that ALT-803 administration via sc injection may result in less toxicity in patients.