Efficacy of ALKS 4230, a Novel Immunotherapeutic Agent, in Murine Syngeneic Tumor Models Alone and in Combination with Immune Checkpoint Inhibitors

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ABSTRACT

ALKS 4230 is a novel immunotherapeutic agent being tested in an ongoing phase 1 study to evaluate safety and tolerability in the treatment of patients with refractory solid tumors. ALKS 4230 is a selective agonist of the intermediate-affinity interleukin 2 (IL-2) receptor that when administered to mice results in a significant increase in numbers of memory CD8$^+$ T cells and NK cells with no expansion of CD4$^+$ regulatory T cells (T_{regs}). The selectivity achieved by ALKS 4230 is due to the fact that the molecule is a fusion of circularly permuted IL-2 to the extracellular portion of the IL-2 receptor α, and the resulting fusion protein is sterically prevented from binding to the high-affinity IL-2 receptor expressed on T_{regs}. The efficacy of ALKS 4230 was compared to recombinant human IL-2 in a B16F10 lung tumor metastasis model. ALKS 4230 treatment resulted in dose-dependent reduction of lung tumor colonization, with 100% inhibition at the highest dose tested. In contrast, while IL-2 was able to reduce lung tumor colonization, the maximal level of inhibition achieved was 60-70% at multiple dose levels such that increasing doses did not result in greater inhibition. Thus, the activation and expansion of effector cells without expansion of T_{regs} in response to ALKS 4230 treatment correlates with its improved efficacy over IL-2, which non-selectively expands both effector cells and T_{regs}. The antitumor efficacy mediated by ALKS 4230 was further evaluated in several murine subcutaneous syngeneic tumor models, including B16F10, MC38 and EMT6. Treatment with ALKS 4230 or its murine ortholog resulted in inhibition of tumor growth and improved survival in multiple models. When dosed in combination with anti-CTLA-4 or anti-PD-1 antibodies, ALKS 4230 resulted in further improvement of tumor growth inhibition and survival. These results demonstrate the murine antitumor efficacy of ALKS 4230 alone and in combination with immune checkpoint inhibitors and support the ongoing clinical evaluation of ALKS 4230 as an immunotherapy for cancer.

METHODS

Potency on Mouse Splenocytes

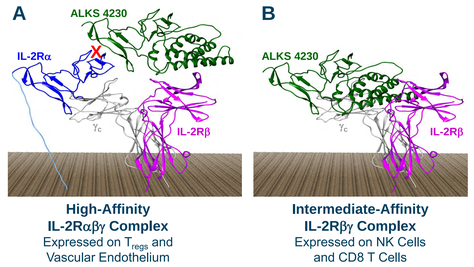
- Mouse splenocytes were isolated by homogenization and RBC lysis and then incubated with ALKS 4230 or rhIL-2 for 30 minutes
- Cells were chemically fixed and stained for surface markers indicative of T_{reg}, NK cell, and memory CD8 T cell populations and then permeabilized and stained for intracellular markers (Foxp3 and pSTAT5)
- Cells were analyzed by flow cytometry on BD LSR Fortessa X-20

Mouse Models of Efficacy

- Unless otherwise noted, all mice were treated in groups of 10.
- Mice were inoculated with the various tumor lines and treated as described in each figure legend. All mice were inoculated at 8-10 weeks of age.
- Tumor volumes were calculated using the following formula: $L \times W^2/2$

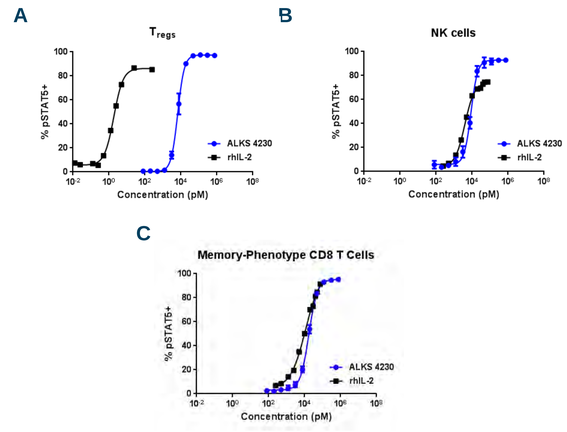
RESULTS

FIGURE 1: ALKS 4230 is Designed to Bind Selectively to the Intermediate-Affinity IL-2 Receptor



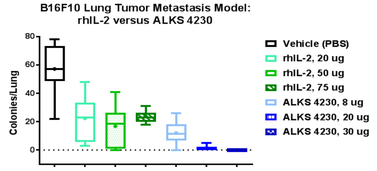
ALKS 4230 is an engineered fusion protein comprised of the extracellular domain of IL-2Rα and circularly permuted IL-2. (**A**) Due to the presence of the IL-2Rα extracellular domain, ALKS 4230 is sterically impaired from binding to the high-affinity IL-2 receptor complex. (**B**) ALKS 4230 retains ability to bind to the intermediate-affinity IL-2 receptor complex. Models are based on the solved crystal complex of IL-2 to the high-affinity IL-2 receptor complex (PDB ID 2B5I).

FIGURE 2: ALKS 4230 Induces STAT5 activation in Murine NK Cells and Memory-Phenotype CD8 T Cells with Similar Potency as rhIL-2, but is 3-4 Orders of Magnitude Less Potent on T_{regs}



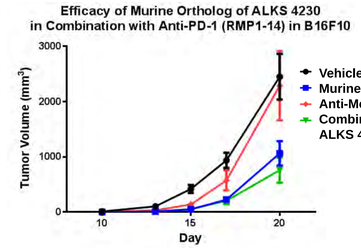
(A) rhIL-2 is 1000-fold more potent on murine T_{regs} relative to ALKS 4230. (B) ALKS 4230 is similarly potent on murine NK cells, and (C) memory CD8 T cells. Potency data generated using CD-1 splenocytes from mixed-gender pools. Data for rhIL-2 are representative of one experiment while data for ALKS 4230 are representative of one experiment with each sample being stimulated in triplicate.

FIGURE 3: Treatment with ALKS 4230 Results in Greater Tumor Inhibition than IL-2 in the B16F10 Lung Metastasis Model



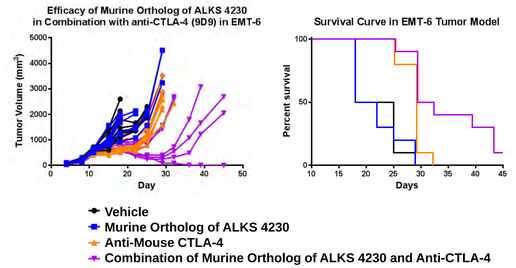
Treatment with ALKS 4230 results in complete inhibition of lung tumor colonization in the murine B16F10 lung metastasis model. 1.5 x 10^5 B16F10 mouse melanoma cells were injected IV into female B6D2F1 mice on Day 1, followed by SC daily dosing of either rhIL-2 (Peprotech) or ALKS 4230 starting day 2 until end of study (Day 11).

FIGURE 4: Treatment with Murine Ortholog of ALKS 4230 Alone and In Combination with Anti-PD-1 in Subcutaneous B16F10 Tumor Model



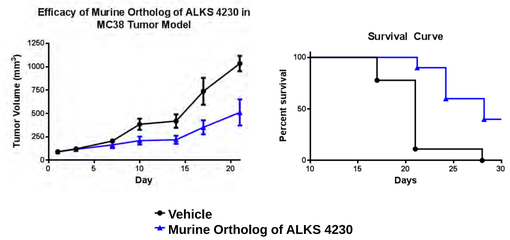
1 x 10^6 B16F10 murine melanoma cells were injected SC into male C57BL/6 mice (n=9/group) on Day 1. The mouse ortholog of ALKS 4230 (1 mg/kg) was administered SC once a day on Days 5-16. Anti-PD-1 (Clone RMP1-14) was administered IP on days 4, 7, 11 and 14 (100 µg/mouse). Results suggest tumor growth inhibition driven primarily by the murine ortholog of ALKS 4230, with some additivity with anti-PD-1.

FIGURE 5: Enhanced Survival After Treatment with Murine Ortholog of ALKS 4230 in Combination with Anti-CTLA-4 in Subcutaneous EMT-6 Tumor Model



5 x 10^6 EMT-6 murine mammary carcinoma cells were injected SC into female Balb/c mice on Day 1. The mouse ortholog of ALKS 4230 (0.8 mg/kg) was administered SC daily on Days 4-8 and 11-15. Anti-CTLA-4 (Clone 9D9) was administered IP on Days 4 (100 µg/mouse), 7 and 10 (50 µg/mouse).

FIGURE 6: Delayed Tumor Growth and Enhanced Survival After Treatment with Murine Ortholog of ALKS 4230 in Subcutaneous MC38 Tumor Model



5 x 10^5 MC38 murine colon adenocarcinoma cells were injected SC into female C57BL/6 mice. Tumors were grown until a tumor volume of ~100 mm^3 (Day 1), and then treatment with the murine ortholog of ALKS 4230 was dosed SC daily at 0.8 mg/kg on days 1-5, 8-12 and 15-19.

CONCLUSIONS

- ALKS 4230 is a novel immunotherapeutic agent that selectively activates the intermediate-affinity IL-2 receptor
- Treatment with ALKS 4230 results in greater anti-tumor efficacy in the mouse B16F10 lung metastasis model relative to rhIL-2
  - ALKS 4230 treatment elicits 100% inhibition at the highest dose tested
  - IL-2 treatment reduces lung tumor colonization, but inhibition plateaus at 60-70%
  - Preferential expansion of T_{regs} by rhIL-2 may limit efficacy
- The murine ortholog of ALKS 4230 delays tumor growth and improves survival in multiple mouse syngeneic tumor models when used as a monotherapy and in combination with the immune checkpoint inhibitors, anti-PD-1 and anti-CTLA-4
- ALKS 4230 is being tested in an ongoing phase 1 study to evaluate safety and tolerability in the treatment of patients with refractory solid tumors

Disclosures: All authors are employees of Alkermes, Inc.

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