A Novel, Individualized Xenograft Model of Cancer Immunotherapy and Tumor Growth Inhibition by ALKS 4230

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INTRODUCTION

Recent successes in tumor immunotherapy highlight the curative potential of modulating patient anti-tumor immune responses. However, preclinical *in vivo* modeling of immune/tumor interactions often depend on a limited number of well-established cell lines. Of available treatment strategies, the use of cytokine therapy offers the advantage of using the patient's own immune cells as anti-tumor effectors. ALKS 4230 is a fusion protein of circularly permuted IL-2 and IL-2 receptor α that is selective for the intermediate-affinity IL-2 receptor expressed on NK cells and subsets of memory and effector T cells. ALKS 4230 is currently in a phase 1 trial to evaluate safety and tolerability in the treatment of patients with refractory solid tumors.

In order to evaluate the ability of ALKS 4230 to promote and enhance anti-human tumor immune responses preclinically, individual xenograft tumor models were established in NOD-*scid* IL2Rgamma^{null} mice (NSG) using tumor cells derived from metastatic melanoma patients following surgical resection. Upon tumor implantation and palpable growth, mice received an adoptive transfer of autologous, unexpanded perhipheral blood mononuclear cells (PBMCs) from the same patient and treatment with ALKS 4230.

We found that autologous T cells successfully engrafted NSG recipient mice after ALKS 4230 treatment similar to IL-2 and that both treatments induced cellular expansion over vehicle controls. Following treatment with ALKS 4230 and adoptive transfer of autologous PBMCs, PDX tumor-bearing mice consistently displayed increased numbers of both CD8 and CD4 T cells migrating into tumor tissue, preferential expansion of non-regulatory T cell subsets, and significant delays in tumor growth as compared to vehicle-treated controls.

Together these data support the rationale for ALKS 4230 as a novel immunotherapeutic for the treatment of melanoma and potentially other solid cancers, as well as the strategy of screening individual, patient-specific xenograft models to assess potential treatment efficacy.

METHODS

Engraftment Studies

- $5x10^6$ PBMCs from normal donors were injected into NOD *scid* IL2R$_{\gamma}^{null}$ (NSG) mice i.v. followed by treatment every 24h with ALKS 4230 (24 µg s.c.), rhIL-2 (50 µg i.p.), or vehicle control for the duration of the experiment.
- After 7 days, spleen, lung, and liver tissues were harvested. Spleen tissue was dissociated through a 40 µM screen, lung and liver tissues were dissociated by tissue-specific enzyme digestion kits (Miltenyi Biotec). Single cell suspensions were treated with a Fc receptor-blocking reagent, incubated with a viability dye, stained with antibodies, and analyzed by flow cytometry.
- During analysis all flow samples were gated on single live cells against a viability dye.

Tumor Dissociation and PDX Establishment

- Melanoma tumor tissue and whole-blood were collected from patients under an IRB-approved protocol.
- Tumor tissue were minced into small fragments and digested into a single-cell suspension with collagenase type IV, hyaluronidase, and DNAse I.
- Resulting cell suspensions were injected subcutaneously into NSG mice in a 75 % Matrigel® matrix and grown for 4-9 weeks.
- Successfully engrafted tumors were harvested at 1000-1200 mm^3, dissociated enzymatically into a single-cell suspension, and cryopreserved until further use.
- PBMCs were enriched from whole blood by centrifugation over a Ficoll-Paque gradient and cryopreserved until subsequent use.

Tumor Injection, Adoptive Transfer, and Treatment

- Cell suspensions from established xenograft tumors were recovered from cryogenesis, injected subcutaneously into NSG mice in a 75 % Matrigel® matrix.
- Once tumors reached 3-4 mm in diameter, mice received $1x10^6$ autologous PBMCs intravenously.
- Drug treatment consisted of 24 µg ALKS 4230 in saline or vehicle control via subcutaneous administration once a day starting the day the mice receive PBMCs until the mice are euthanized.

Tumor Measurement and Immune Infiltrate Analysis

- Tumor volumes were calculated using the following formula: $V = (L \times W^2)/2$
- Spleen tissues were dissociated through a 40 µM screen prior to staining with antibodies for flow cytometric analyses.
- Tumor tissues were dissociated by collagenase/hyaluronidase/DNAse I digestion prior to staining with antibodies for flow cytometric analyses.

RESULTS

FIGURE 1: ALKS 4230 enhances expansion of adoptively transferred PBMCs in NSG mice similar to IL-2.

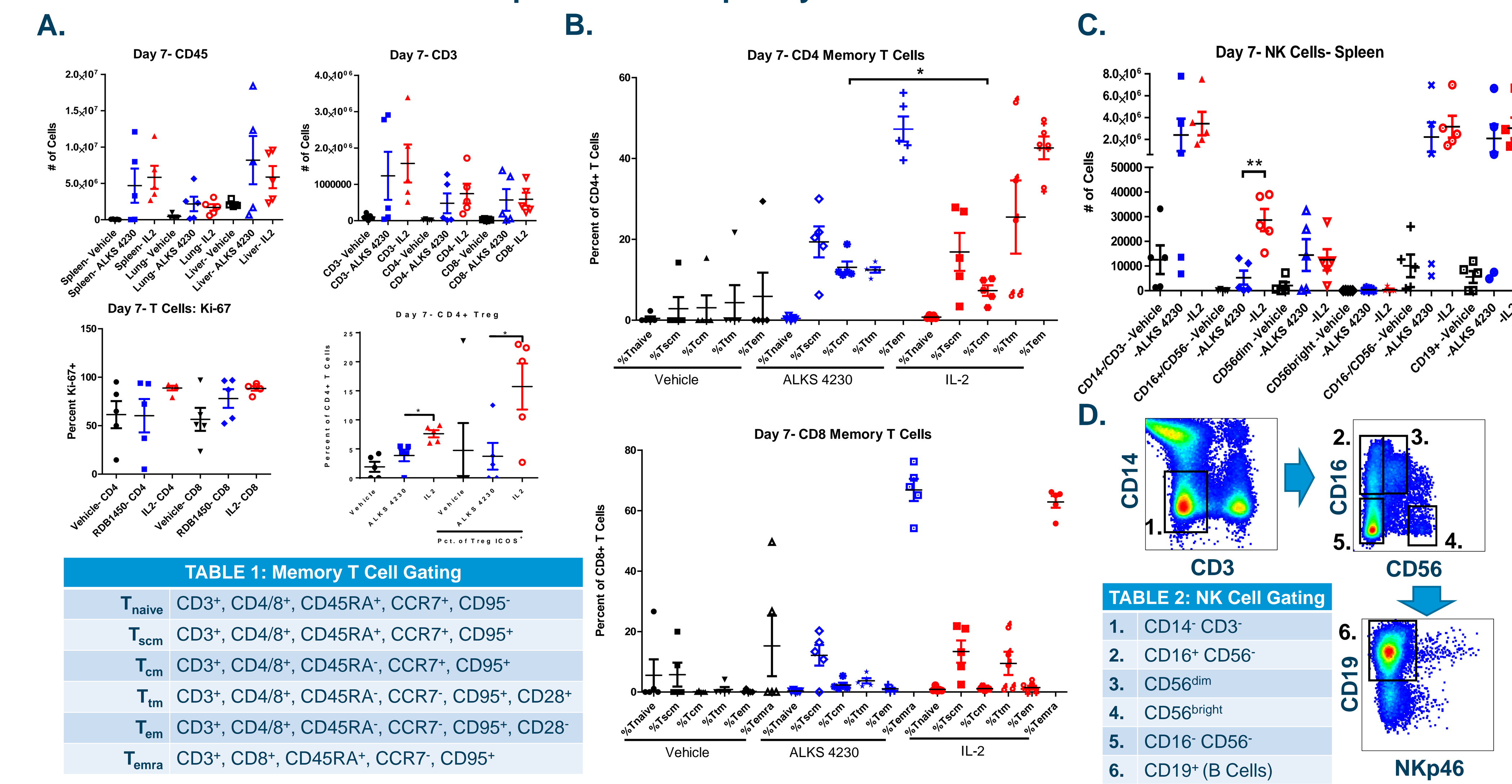
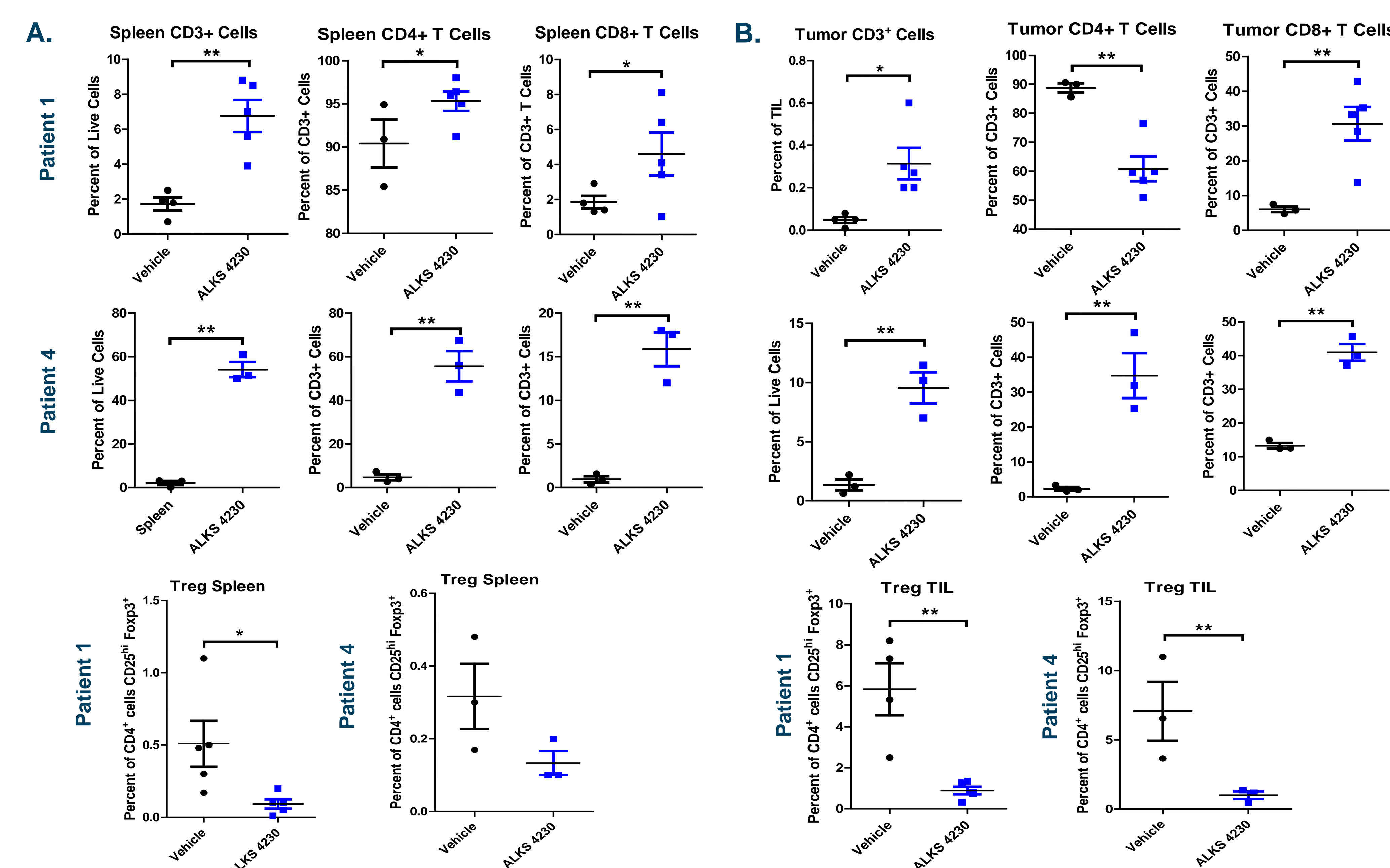


TABLE 1: Memory T Cell Gating	
T_{naive}	$CD3^+$, $CD4/8^+$, $CD45RA^+$, $CCR7^+$, $CD95^-$
T_{scm}	$CD3^+$, $CD4/8^+$, $CD45RA^+$, $CCR7^+$, $CD95^+$
T_{cm}	$CD3^+$, $CD4/8^+$, $CD45RA^-$, $CCR7^+$, $CD95^+$
T_{tm}	$CD3^+$, $CD4/8^+$, $CD45RA^-$, $CCR7^-$, $CD95^+$, $CD28^+$
T_{em}	$CD3^+$, $CD4/8^+$, $CD45RA^-$, $CCR7^-$, $CD95^+$, $CD28^-$
T_{emra}	$CD3^+$, $CD8^+$, $CD45RA^+$, $CCR7^-$, $CD95^+$

TABLE 2: NK Cell Gating	
1.	$CD14^-$ $CD3^-$
2.	$CD16^+$ $CD56^-$
3.	$CD56^{dim}$
4.	$CD56^{bright}$
5.	$CD16^-$ $CD56^-$
6.	$CD19^+$ (B Cells)

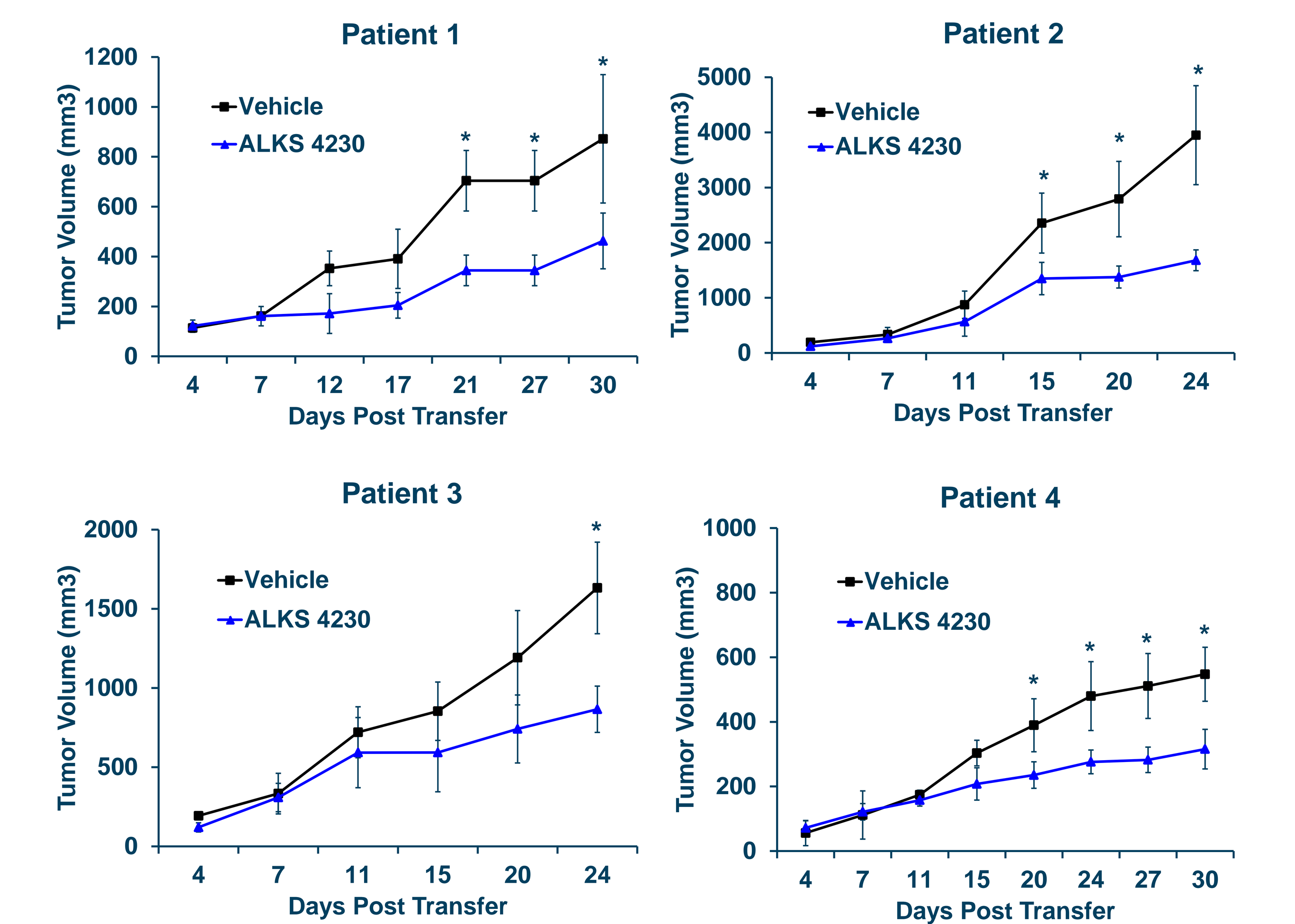
$5x10^6$ PBMCs from a normal donor were adoptively transferred into NSG mice. Animals then received ALKS 4230 (24 µg s.c.), rhIL-2 (50 µg i.p.), or vehicle daily for seven days after which spleen, liver, and lung tissues were harvested and digested. T and NK cell engraftment were then assessed by flow cytometry. A.) Assessment of transferred lymphocyte (CD45+) and T cell populations. T_{regs} were defined as $CD4^+$ $CD25^{high}$ $FOXP3^+$. B.) CD4 and CD8 T cell subsets were further defined according to expression of memory-associated markers as defined in Table 1. *p = <0.05 for $CD4^+$ Tcm cells in ALKS 4230 vs rhIL-2 treatment groups. C.) NK cell populations assessed according to the indicated markers. D.) A representative PBMC sample demonstrating the gating strategy for NK and B cell populations shown in C and defined in Table 2. **p = <0.01 for $CD16^+$ $CD56^-$ cells in ALKS 4230 vs rhIL-2 groups. Statistical significance was determined using the unpaired t test.

FIGURE 2: ALKS 4230 treatment results in peripheral expansion and infiltration of CD8 and non-T_{reg} CD4 T cells into tumor tissue.



Tumor and spleen tissues were harvested from animals injected with PDX tumors and autologous PBMCs followed by treatment with vehicle or ALKS 4230. T cell subsets from spleen A.) and tumor B.) were then evaluated by flow cytometry. *p = <0.05 and **p = <0.01 for the ALKS 4230 vs. vehicle control group determined using an unpaired t test.

FIGURE 3: ALKS 4230 treatment significantly delays tumor growth in NSG mice receiving an adoptive transfer of autologous PBMCs.



Tumors derived from four melanoma patients were implanted into NSG mice, followed by adoptive transfer of autologous PBMCs and subsequently treated once a day with vehicle or ALKS 4230. Graphs indicate tumor volumes calculated according to $V = (L \times W^2)/2$.
* p < 0.05 for ALKS 4230 vs. the vehicle control group determined using an unpaired t test at the indicated time points.

CONCLUSIONS

- ALKS 4230 and rhIL-2 treatment similarly supported the engraftment of human PBMCs in non-tumor bearing NSG recipient mice.
- rhIL-2, but not ALKS 4230, induced the expansion of $ICOS^+$ T_{regs} in non-tumor bearing NSG mice.
- ALKS 4230 treatment induced the expansion of non-T_{reg} $CD4^+$ T cells and $CD8^+$ T cells in both peripheral and tumor tissues.
- ALKS 4230 treatment led to increased infiltration of both $CD4^+$ and $CD8^+$ T cells into tumor tissue.
- ALKS 4230 treatment in mice induced significant growth delay of melanoma tumors derived from 4 different individuals after surgical de-bulking.

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