

# Selective ITK blockade induces antitumor responses and enhances efficacy to immune checkpoint inhibitors in preclinical models

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## Abstract

Interleukin 2 inducible T cell kinase (ITK) plays a role in both T cell receptor (TCR) signaling and T helper cell differentiation. ITK<sup>-/-</sup> mice exhibit defects in Th2 differentiation while retaining the ability to differentiate into Th1 cells that secrete IFN $\gamma$ . To study the function of ITK, we generated CPI-818, a covalent inhibitor of ITK (K<sub>D</sub> 2.5nM) with > 100 fold selectivity over resting lymphocyte kinase (RLK) and other TEC family kinases. We have investigated the immunomodulatory effects of specific ITK blockade of human T cells with CPI-818 in vitro and in vivo in murine tumor models. Analysis of the cytokine profiles in human CD4 T cells differentiated under non-polarizing, Th1 or Th2 polarizing conditions showed that CPI-818 inhibited generation of Th2-associated cytokines whereas, production of Th1 cytokines such as IFN $\gamma$  were largely unaffected. Suppression of IL-2-mediated proliferative response occurred only at high concentrations of CPI-818 (>1 $\mu$ M). The effect of CPI-818 on anti-tumor immunity was evaluated in both murine syngeneic CT26 colon cancer and EL4 T cell lymphoma models. In vivo treatment of animals with established tumors showed single-agent anti-tumor activity in both models. Combining CPI-818 with suboptimal doses of anti-PD1 and anti-CTLA4 synergistically inhibited the growth of established CT26 tumors, leading to complete tumor elimination in 100% treated animals. Furthermore, triple combination therapy elicited durable anti-tumor immune memory after animals were rechallenged with a new engraftment of CT26 cells. Enhancement of anti-tumor activity required CD8+ T cells as in vivo antibody-mediated depletion of CD8, but not CD4, abolished the efficacy of CPI-818 monotherapy. Consistent with that, we found increased tumor-infiltrating CD8+ T cells and increased ratio of CD8: Treg in the responding tumors of CPI-818 treated animals. Levels of several exhaustion makers were down-regulated by treatment with CPI-818, suggesting that inhibition of ITK by CPI-818 produces favorable changes in the tumor microenvironment. Taken together, our findings provide insights into the consequences of selective ITK blockade representing a potential novel approach to immunotherapy of cancer.

## Background

### (A) CPI-818 selectively inhibits ITK

### (B) ITK function in CD4 T helper cell differentiation

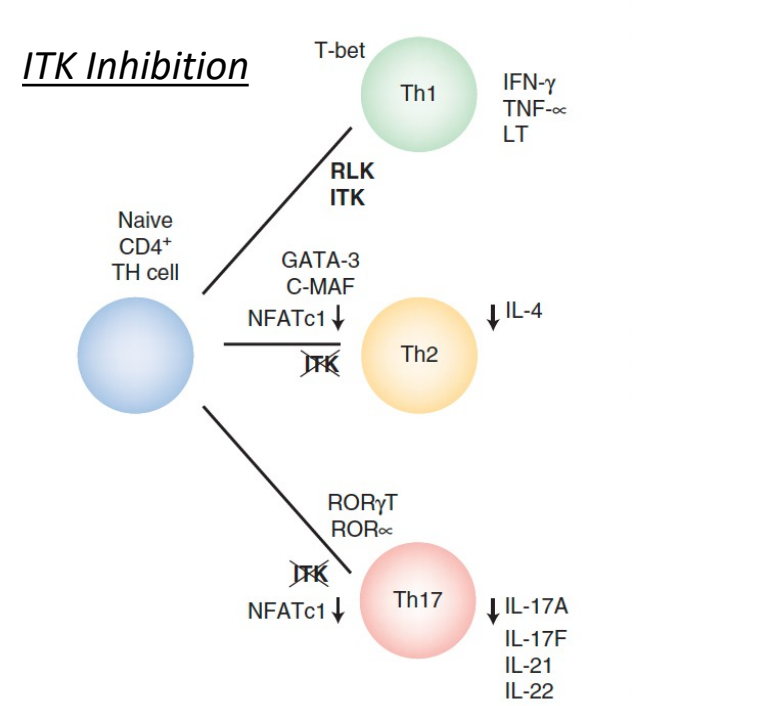
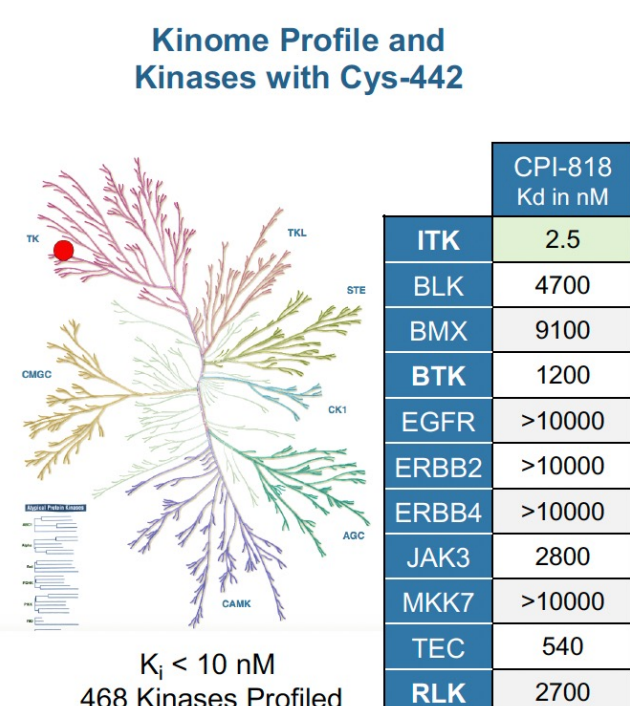


Figure adapted from *Cold Spring Harb Perspect Biol* 2010;2:a002287

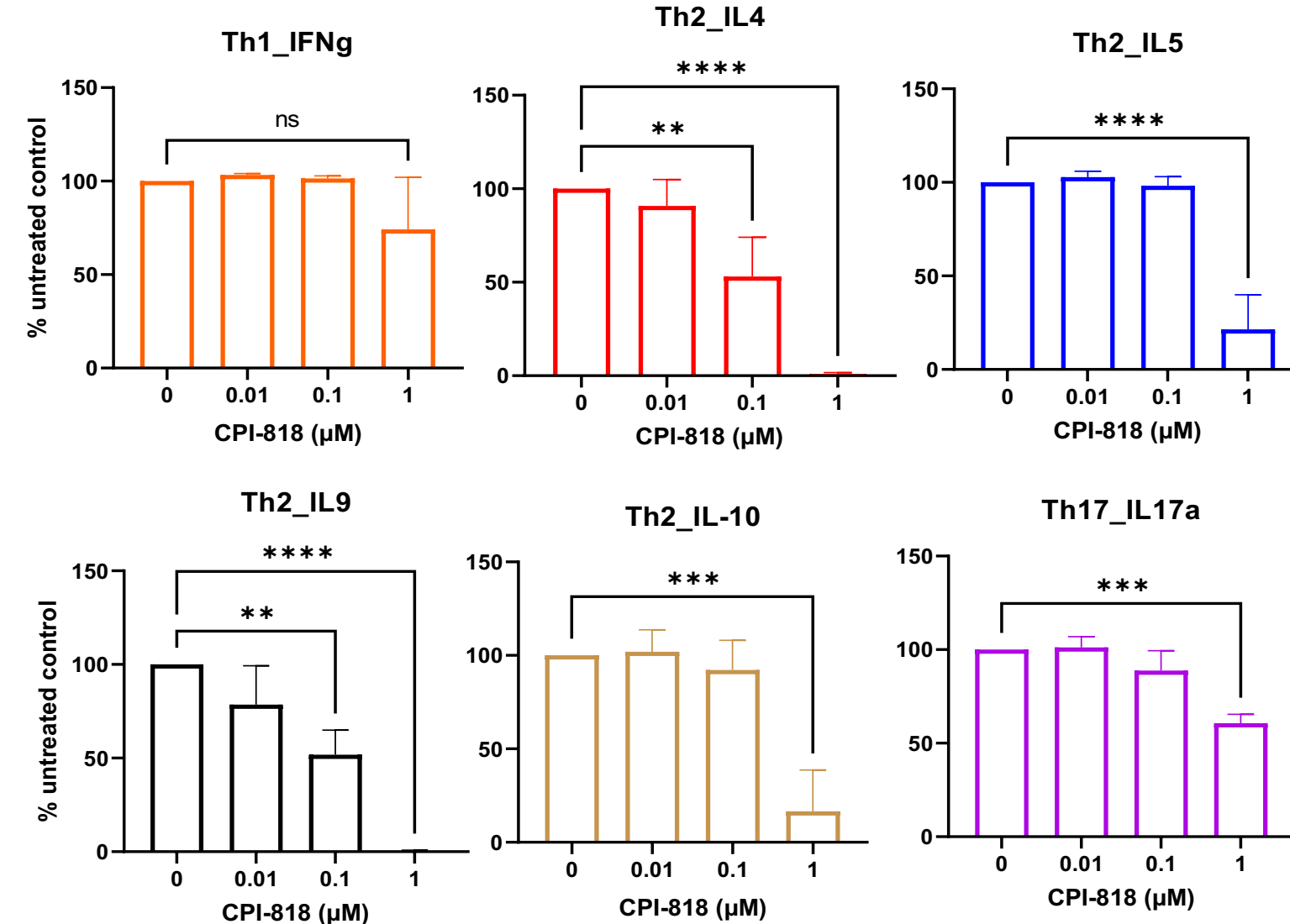
## Methods

**Culture of polarized CD4 T helper (Th) cells:** Purified human naïve CD4+ T cells (CD45RA+CD25-) from healthy donors were stimulated by immobilized anti-CD3/CD28 antibodies in the presence of in Th polarizing cocktails for 6 days and restimulated again with or without CPI-818 for 48 hrs. Culture supernatants were collected and the amounts of cytokines were assessed by MSD. (Th1 condition: human IFN $\gamma$  (10 ng/ml), IL-12 (10 ng/ml), anti-IL-4 (10  $\mu$ g/ml); Th2 condition: human IL-4 (10 ng/ml), IL-5 (10 ng/ml), anti-IFN $\gamma$  (10  $\mu$ g/ml); Th17 condition: human IL-6 (10 ng/ml), IL-1b (10 ng/ml), IL-23 (10 ng/ml), TGF $\beta$  (10 ng/ml), anti-IL-4 (10  $\mu$ g/ml), anti-IFN $\gamma$  (10  $\mu$ g/ml).

**Syngeneic murine tumor models:** Murine cancer cell lines such as CT26 (Colorectal carcinoma), B16F10-OVA (Melanoma), RENCA (Renal cell carcinoma), EL4 (T cell lymphoma), A20 (B cell lymphoma) were subcutaneously implanted to either Balb/c or C57BL/6 mice. Mice with established tumors (75-100 mm<sup>3</sup>) were treated with CPI-818 in either chow or solution formulation alone as monotherapy or in combination with anti-PD1 (clone RPM1-1, 25  $\mu$ g/mouse) and anti-CTLA4 (clone 9H10, 25  $\mu$ g/mouse).

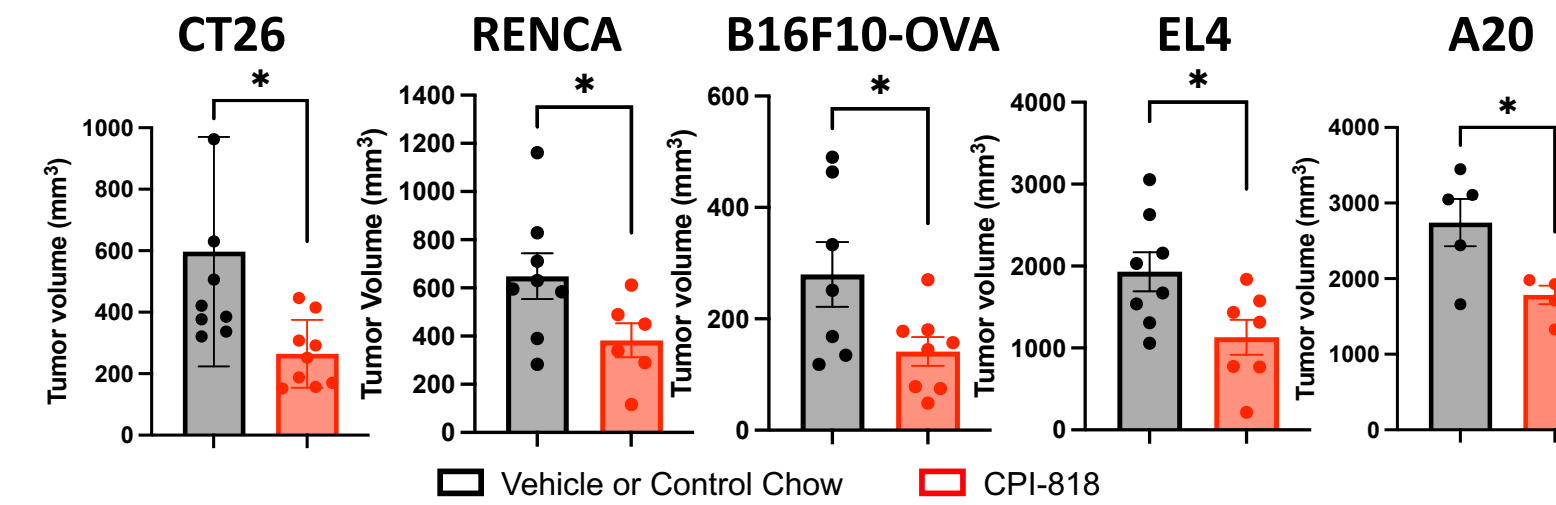
**Statistics:** Analyses were performed in GraphPad Prism software. ns > 0.05, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001.

## CPI-818 Preferentially Inhibits Th2 Cytokines in Differentiated Human T Helper Cells



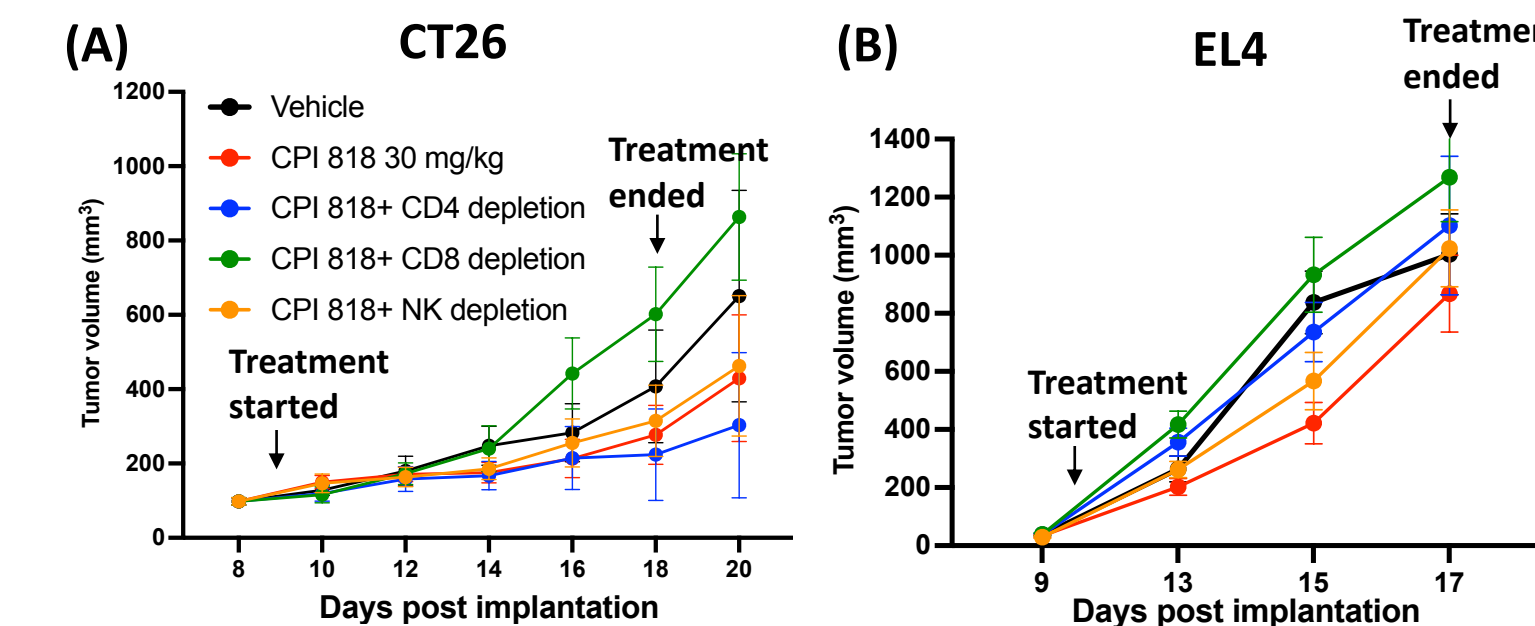
**Fig 1. CPI-818 suppresses Th2-associated cytokine production in polarized Th2 cells.** Purified naïve CD4 T cells from three donors were first polarized under Th1, Th2, and Th17 conditions for 6 days before reactivated in the presence of increasing concentrations of CPI-818 as indicated or DMSO alone. The cytokine concentrations from DMSO-treated samples (untreated control) are set to 100%. Then the concentrations in stimulated samples treated with CPI-818 are expressed as % of those in stimulated samples treated with DMSO alone (untreated control). Data were shown as mean  $\pm$  SEM. P values were determined using ordinary one-way ANOVA.

## CPI-818 Single Agent is Active in Several Preclinical Mouse Tumor Models



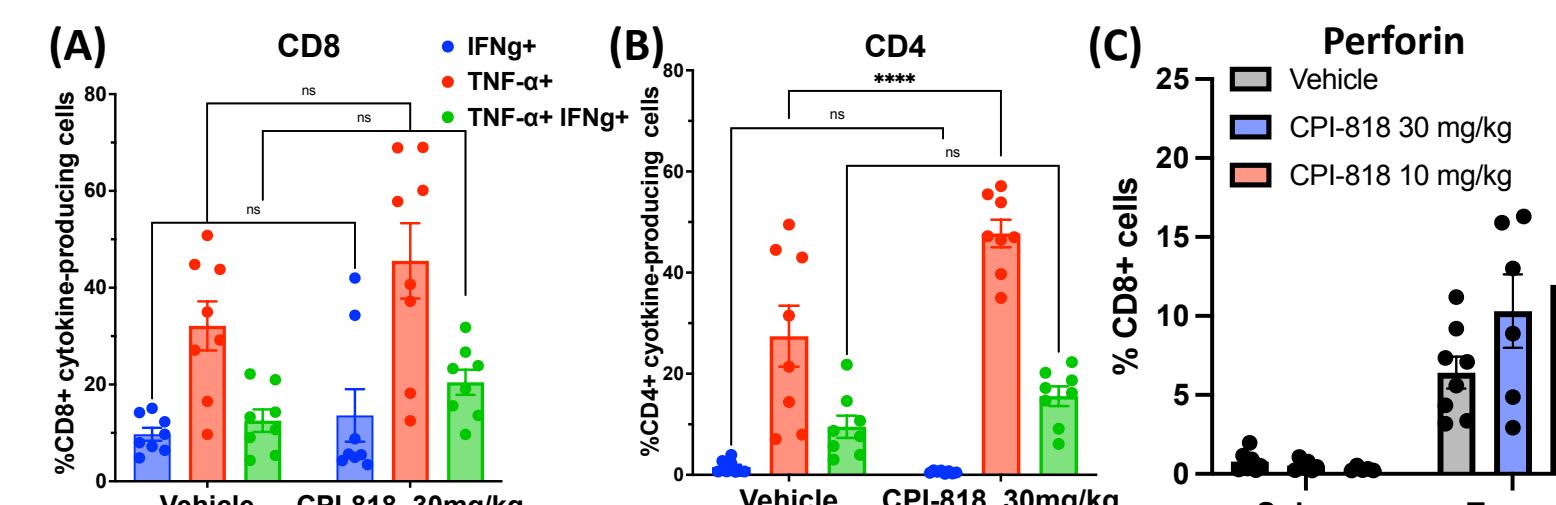
**Fig 2. Anti-tumor activity of CPI-818 as single agent across a range of syngeneic murine tumor models.** Mice with established tumors indicated here were treated with vehicle/control chow or CPI-818, 30 mg/kg (CT26, & EL4), 10 mg/kg (RENCA, & B16F10-OVA) or CPI-818 chow, 130 mg/kg (A20) daily for 7-8 days (all tumors except A20) or for 13 days (A20). Tumor measurements were performed 2-3 days after last dosing. P values were determined using two-tailed unpaired t test.

## T cells and Natural Killer Cells Contribute to the Anti-tumor Effects of CPI-818



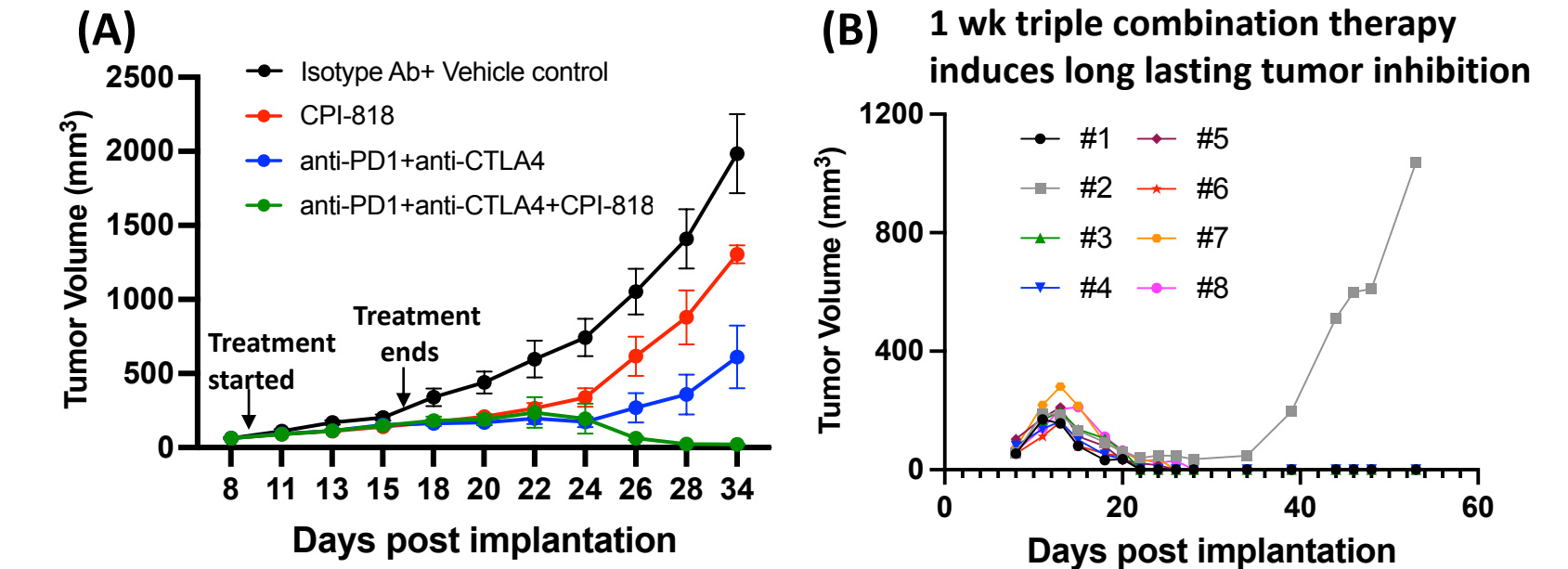
**Fig 3. T cell and/or NK cell depletion increases tumor growth in CPI-818-treated mice.** (A) Depletion of CD8+ T cells abolishes antitumor efficacy in the CT26 tumor model. (B) Host CD4+, CD8+ and NK cells are required for antitumor effects of CPI-818 in the EL4 tumor model. Antibody depletion started one day prior to vehicle or CPI-818 (30mg/kg) treatment. Mice (n=7-8/group) were treated via oral gavage with CPI-818 or vehicle control for 8 days (A) or 7 days (B). Anti-CD4 (clone GK1.5, 200  $\mu$ g/mouse), anti-CD8 (clone 53-6.72, 200  $\mu$ g/mouse), anti-NK1.1 (clone PK136, 200  $\mu$ g/mouse) antibodies were used for depletion. Data shown as the mean  $\pm$  SEM of 7-8 mice/group.

## CPI-818 Increases Cytolytic Capacity of TILs



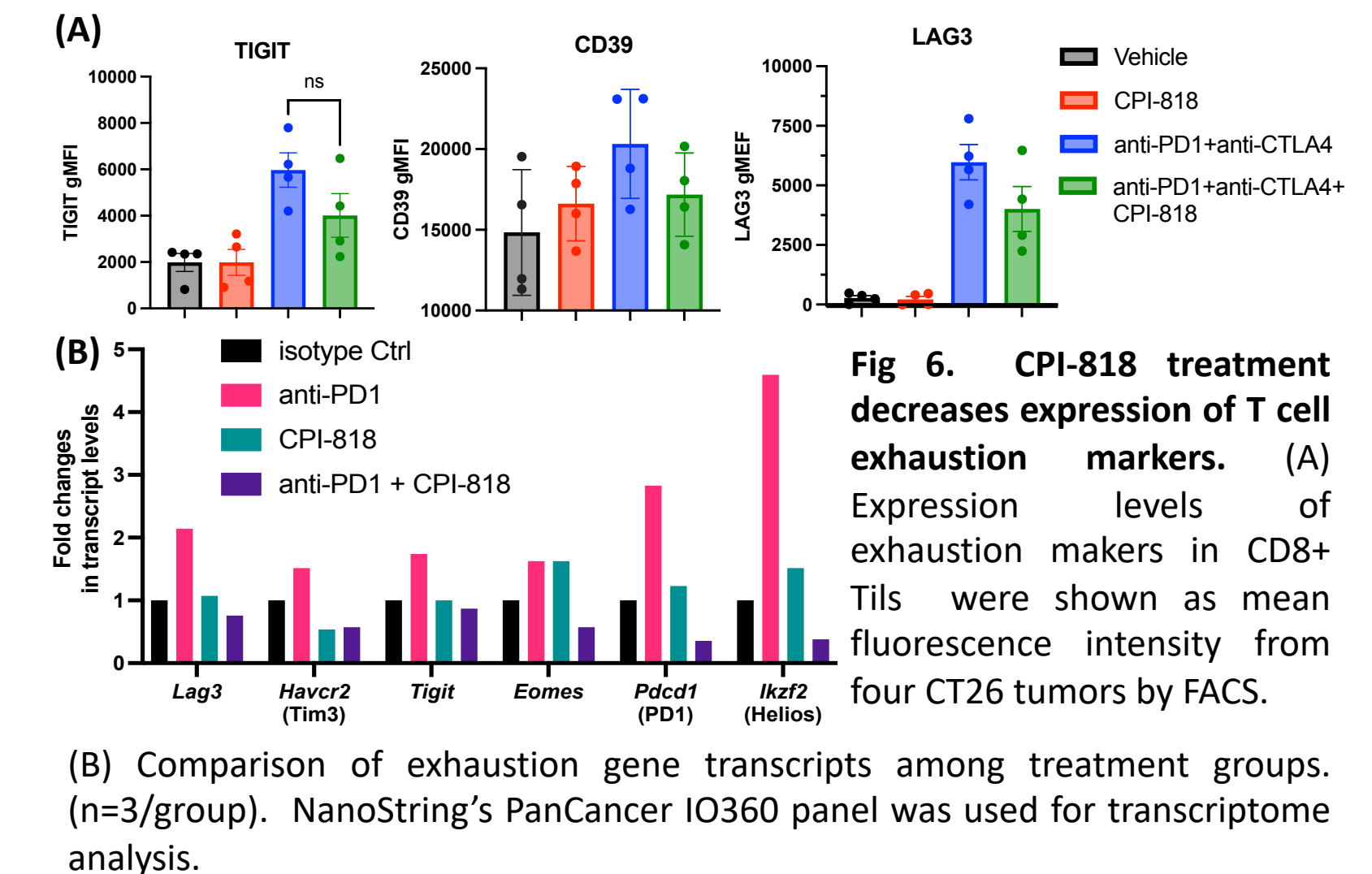
**Fig 4. CPI-818-treated mice show increased production of proinflammatory cytokines and perforin that are associated with cytolytic activity of T cells in tumors.** (A-C) Detection of IFN $\gamma$ , TNF $\alpha$ , and perforin-producing T cells in CT26 (A&B) and EL4 models (C) by intracellular staining after TILs were stimulated with PMA and ionomycin for 4hr.

## CPI-818 Enhances anti-PD1 and anti-CTLA4 Efficacy in CT26 Tumor Model



**Fig 5. CPI-818 in combination with anti-PD1 and anti-CTLA4 induces long-term anti-tumor immunity.** (A) CPI-818 synergizes with anti-PD1 and anti-CTLA4 treatment. Treatment with suboptimal dose (25  $\mu$ g/mouse) of anti-PD1 and anti-CTLA4 resulted in incomplete inhibition of tumor growth, whereas triple combination treatment with CPI-818 (30 mg/kg) led to durable anti-tumor response even after treatment was terminated. Data shown as the mean  $\pm$  SEM of 8 mice per group. (B) Spider plots of individual animals from the triple combination treatment group in one representative study. 19 out of 20 mice treated with CPI-818 showed complete tumor regression from three studies.

## Combination of CPI-818 with Checkpoint Inhibitor Blockade Reduces T cell Exhaustion in Tumors



**Fig 6. CPI-818 treatment decreases expression of T cell exhaustion markers.** (A) Expression levels of exhaustion makers in CD8+ Tils were shown as mean fluorescence intensity from four CT26 tumors by FACS. (B) Comparison of exhaustion gene transcripts among treatment groups. (n=3/group). NanoString's PanCancer IO360 panel was used for transcriptome analysis.

## Conclusions

- CPI-818 is a potent and selective ITK inhibitor that spares RLK.
- CPI-818 preferentially blocks Th2 cytokine production in polarized T helper cells.
- CPI-818 monotherapy is active in several murine tumor models such as CT26, RENCA, B16F10, EL4 and A20.
- Combination of CPI-818 with anti-PD1 and anti-CTLA4 is synergistic, resulting in complete tumor elimination in 19 out of 20 tumor-bearing mice tested.
- T cells and NK cells contribute to CPI-818-mediated antitumor activity.
- CPI-818 enhances cytolytic capacity of CD8+ TILs.
- CPI-818 reduces T cell exhaustion marker expression and increases T cell stemness in the TME.
- CPI-818's multiple immune effects may offer an appealing new approach to immunotherapy of cancer.