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

Lih-Yun Hsu[1], Rahul Pawar[1], Dan Li[1], Poorva Ghosh[1], Kevin Nguyen[1], Carlene Williams[1], Yuqin Song[2], Ning Ding[2], Erik Verner[3], and Richard Miller[1,3]
[1]Corvus Pharmaceuticals, Inc, Burlingame, CA, USA; [2]Peking University Cancer Hospital & Institute, Beijing, China; [3]Angel Pharmaceuticals Co., Ltd, Jiaxing, China

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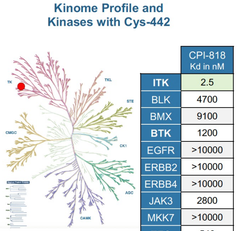
Abstract

Interleukin 2 inducible T cell kinase (ITK) plays a role in both T cell receptor (TCR) signaling and T helper cell differentiation. ITK-/- mice exhibit defects in Th2 differentiation while retaining the ability to differentiate into Th1 cells that secrete IFNγ. To study the function of ITK, we generated CPI-818, a covalent inhibitor of ITK (K_D 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 IFNg were largely unaffected. Suppression of IL-2-mediated proliferative response occurred only at high concentrations of CPI-818 (>1μM). 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



	CPI-818 Kd in nM
ITK	2.5
BLK	4700
BMX	9100
BTK	1200
EGFR	>10000
ERBB2	>10000
ERBB4	>10000
JAK3	2800
MKK7	>10000
TEC	540
RLK	2700

(B) ITK function in CD4 T helper cell differentiation

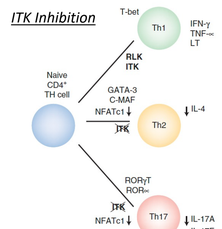


Figure adapted from *Cold Spring Harb Perspect Biol 2010;2:a002287*

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 IFNγ (10 ng/ml), IL-12 (10 ng/ml), anti-IL-4 (10 ug/ml); Th2 condition: human IL-4 (10 ng/ml), IL-5 (10 ng/ml), anti-IFNγ (10 μg/ml); Th17 condition: human IL-6 (10 ng/ml), IL-1b (10 ng/ml), IL-23 (10 ng/ml), TGFβ (10 ng/ml), anti-IL-4 (10 μg/ml), anti-IFNγ (10 μg/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^3) were treated with CPI-818 in either chow or solution formulation alone as monotherapy or in combination with anti-PD1 (clone RPM1-1, 25 μg/mouse) and anti-CTLA4 (clone 9H10, 25 μg/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

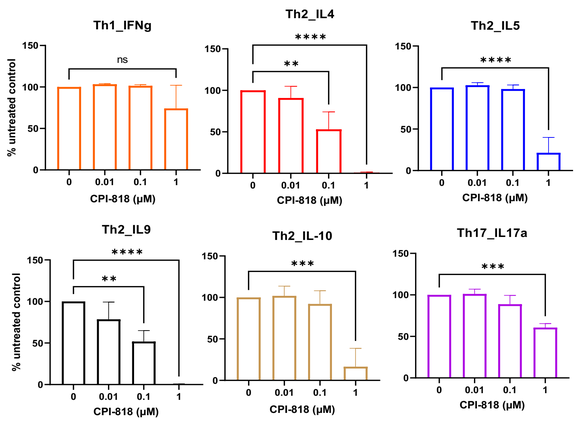


Fig 1. CPI-818 suppresses Th2-associated cytokine production in polarized Th2 cells. Purified naïve CD4 T cells from three donors were first polarized under Th1, Th2, and Th17 conditions for 6 days before reactivated in the presence of increasing concentrations of CPI-818 as indicated or DMSO alone. 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 ± SEM. P values were determined using ordinary one-way ANOVA.

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

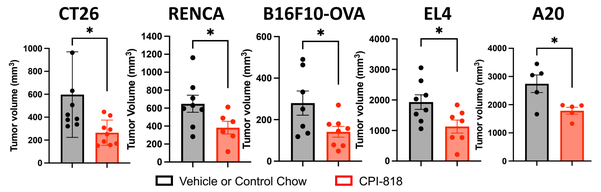


Fig 2. Anti-tumor activity of CPI-818 as single agent across a range of syngeneic murine tumor models. Mice with established tumors indicated here were treated with vehicle/control chow or CPI-818, 30 mg/kg (CT26, & EL4), 10 mg/kg (RENCA, & B16F10-OVA) or CPI-818 chow, 130 mg/kg (A20) daily for 7-8 days (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

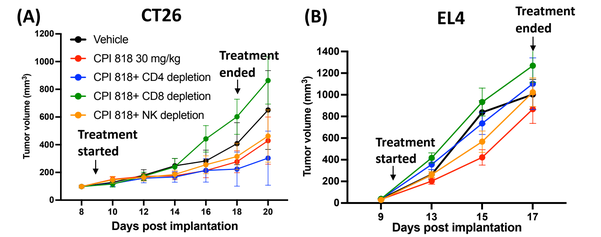


Fig 3. T cell and/or NK cell depletion increases tumor growth in CPI-818-treated mice. (A) Depletion of CD8+ T cells abolishes antitumor efficacy in the CT26 tumor model. (B) Host CD4+, CD8+ and NK cells are required for antitumor effects of CPI-818 in the EL4 tumor model. Antibody depletion started 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 ug/mouse), anti-CD8 (clone 53-6.72, 200 ug/mouse), anti-NK1.1 (clone PK136, 200 ug/mouse) antibodies were used for depletion. Data shown as the mean ± SEM of 7-8 mice/ group.

CPI-818 Increases Cytolytic Capacity of TILs

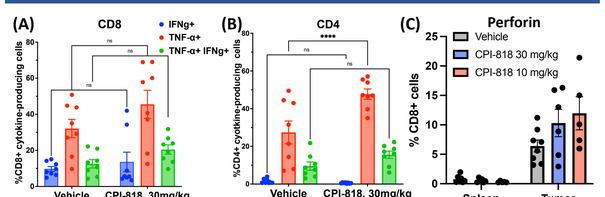


Fig 4. CPI-818-treated mice show increased production of proinflammatory cytokines and perforin that are associated with cytolytic activity of T cells in tumors. (A-C) Detection of IFNγ, TNFα, and perforin-producing T cells in CT26 (A&B) and EL4 models (C) by intracellular staining after TILs were stimulated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

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

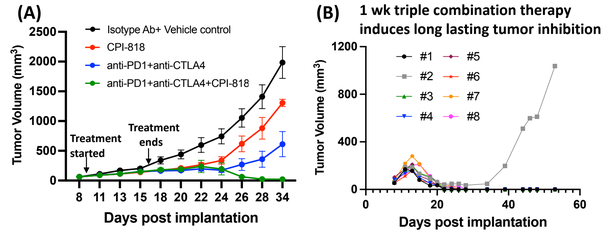


Fig 5. CPI-818 in combination with anti-PD1 and anti-CTLA4 induces long-term anti-tumor immunity. (A) CPI-818 synergizes with anti-PD1 and anti-CTLA4 treatment. Treatment with suboptimal dose (25 μg/mouse) of anti-PD1 and anti-CTLA4 resulted in incomplete inhibition of tumor growth, whereas triple combination treatment with CPI-818 (30 mg/kg) led to durable anti-tumor response even after treatment was terminated. Data shown as the mean ± 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

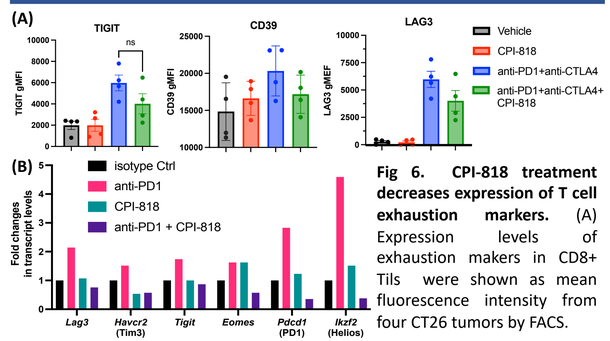


Fig 6. CPI-818 treatment decreases expression of T cell exhaustion markers. (A) Expression levels of exhaustion makers in CD8+ Tils were shown as mean fluorescence intensity from four CT26 tumors by FACS.

(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.