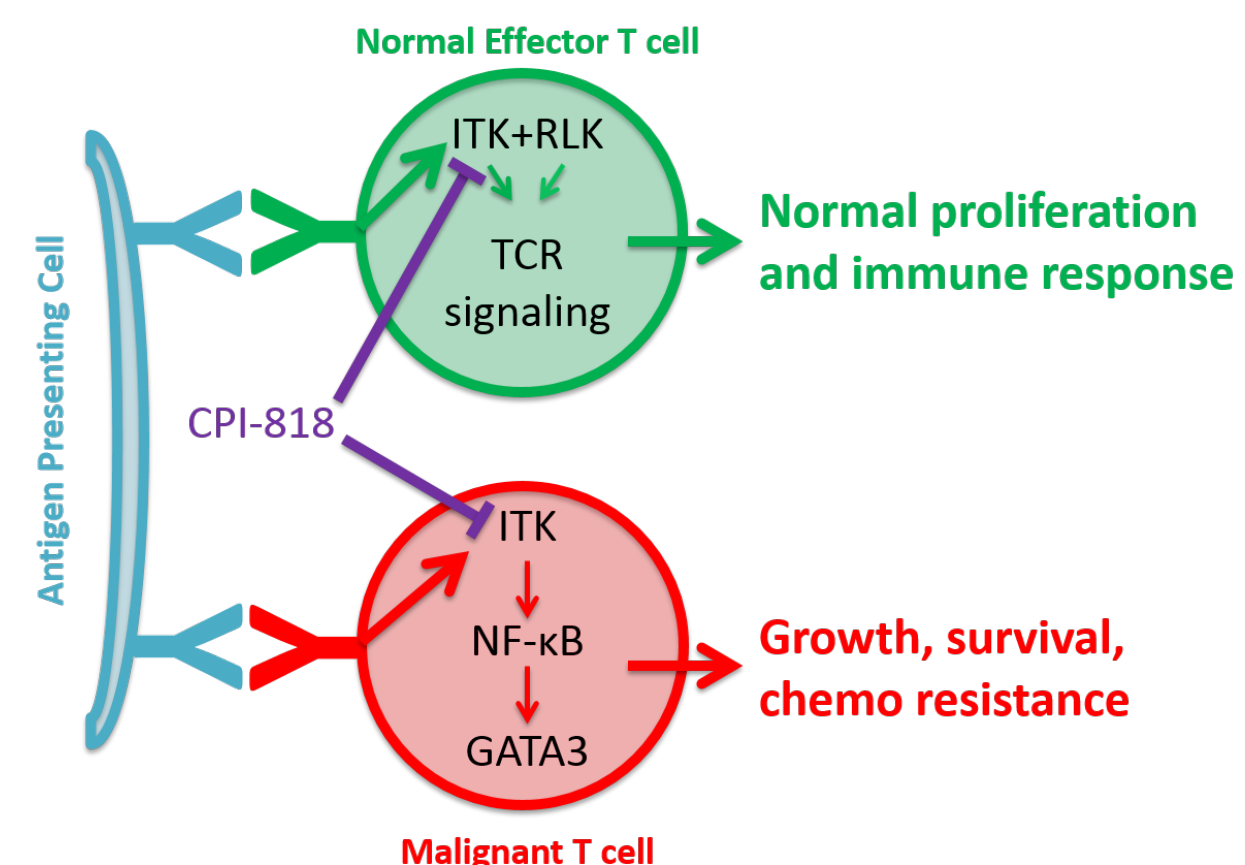


Introduction

- T-cell lymphomas (TCL) comprise a rare, aggressive, and heterogeneous subtype of non-Hodgkin lymphoma
- Outcomes for patients with TCL remain poor and novel therapies are needed
- Engagement of the T-cell receptor (TCR) in malignant T-cells leads to interleukin-2-inducible T-cell kinase (ITK) dependent activation of NF- κ B and GATA3, and promotes chemotherapy resistance¹.
- TCR-mediated chemotherapy resistance is reversed upon knockdown or inhibition of ITK¹.
- ITK and resting lymphocyte kinase (RLK) are partially redundant TEC family kinase members involved in TCR signaling².
- Here, we report results from preclinical studies evaluating the use of the ITK-specific inhibitor CPI-818 (ITKi) and the dual ITK/RLK inhibitor CPI-893 (ITK/RLKi) in the treatment of T-cell lymphoproliferative disorders.

Visual Abstract



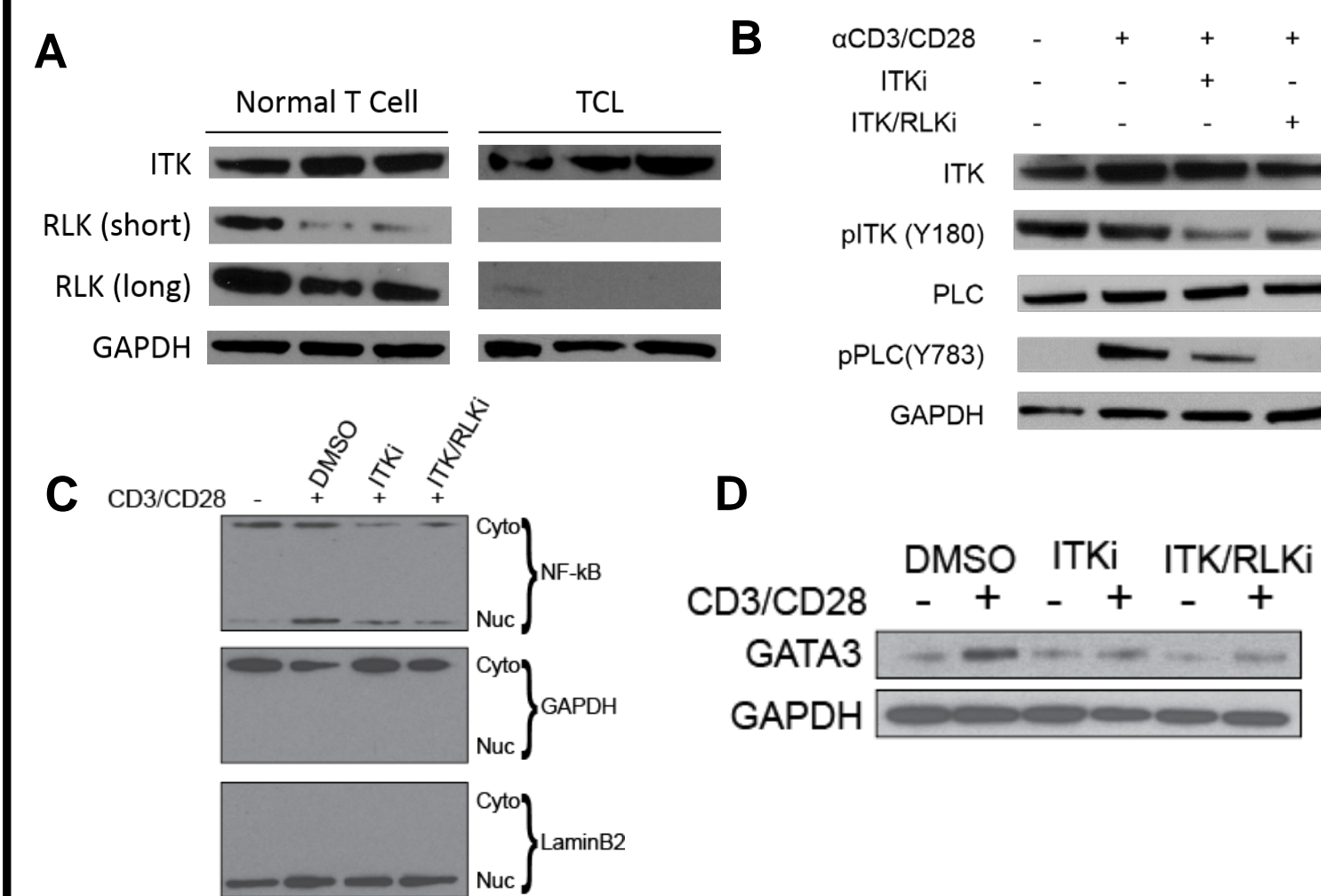
Results and Methods

Table 1: CPI-818 specifically inhibits ITK

	IC50 (nM)	
	ITK	RLK
CPI-818 (ITKi)	2.3	260
CPI-893 (ITK/RLKi)	0.36	0.4

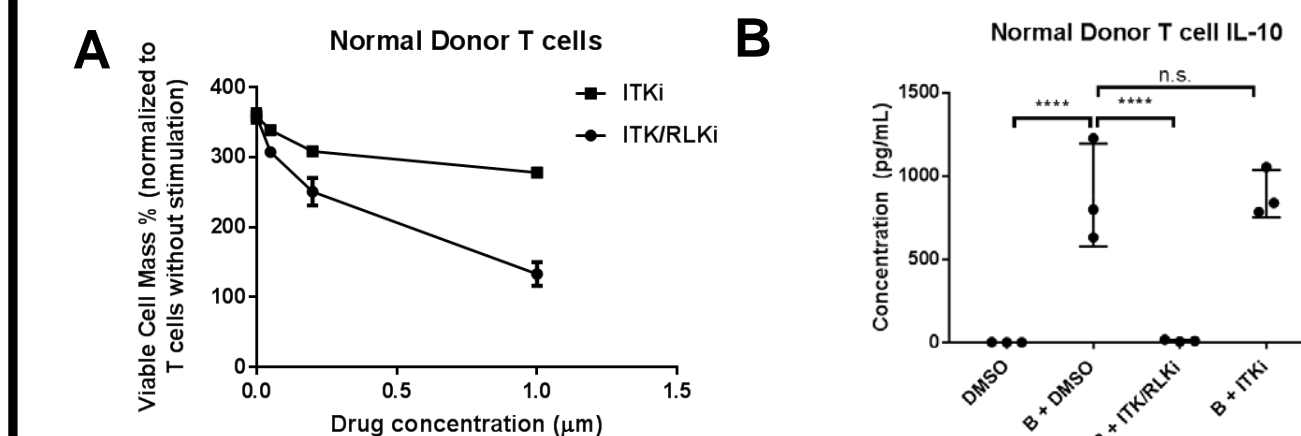
Kinome screening was performed for CPI-818 (ITKi) and CPI-893 (ITK/RLKi). CPI-818 (ITKi) had high specificity for ITK over RLK (IC50 2.3 nM and 260 nM, respectively). In contrast CPI-893 (ITK/RLKi) had a high affinity for both ITK and RLK (IC50 0.36 nM and 0.4 nM, respectively)

Figure 1: CPI-818 and CPI-893 inhibit TCR signaling



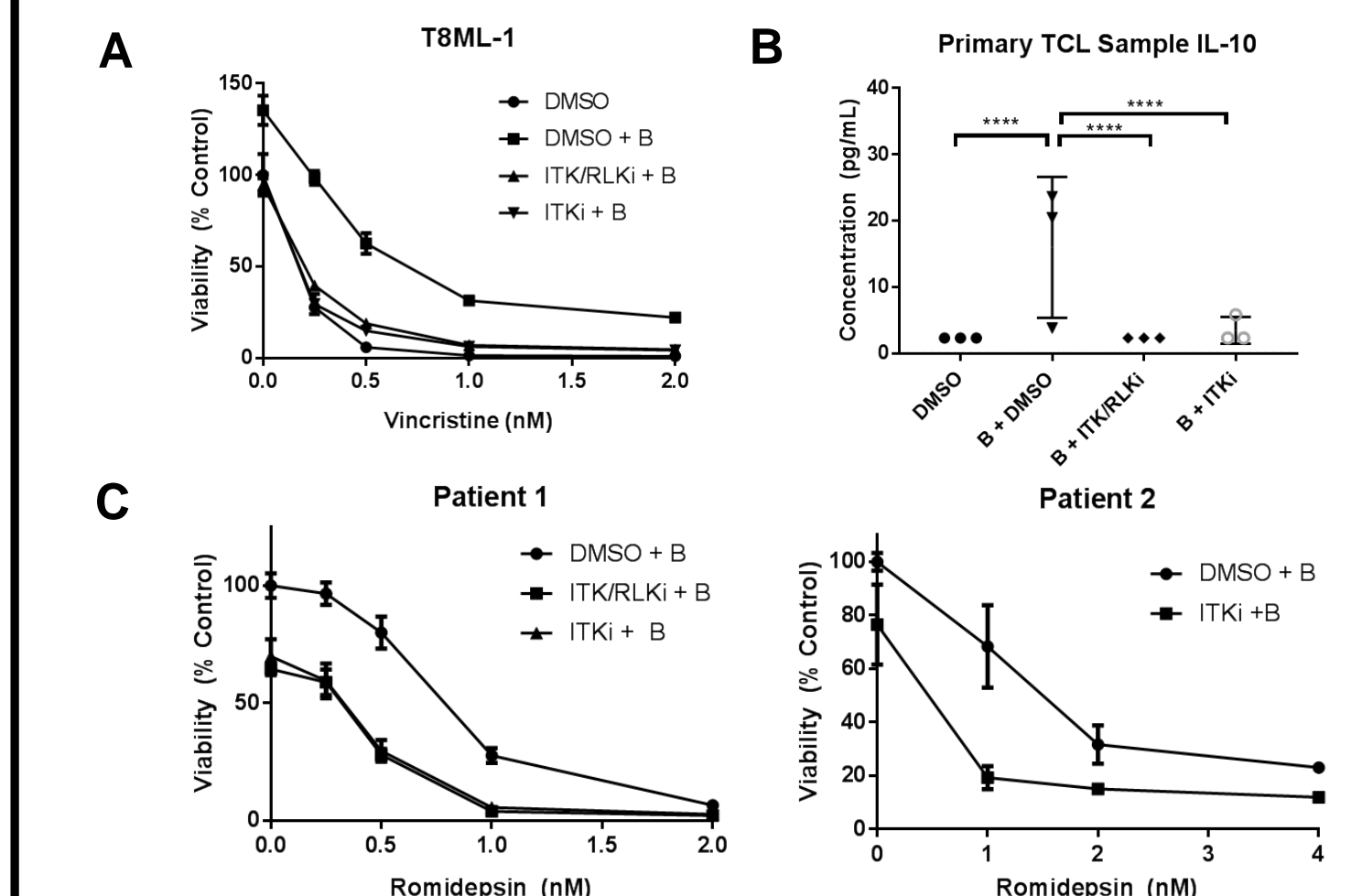
- (A) Normal human T cells from healthy donors and malignant T cells from patients with peripheral blood involvement by TCL were purified using CD3 positive selection followed by protein isolation and western blotting. Normal T cells expressed both ITK and RLK (n=3/3) while malignant T cells preferentially expressed ITK (n=3/3) and low level RLK in a single case that was seen only on overexposure of the blot ["RLK(long)"].
- (B) Jurkat cells (express ITK, but not RLK) were stimulated with anti-CD3/CD28 beads for 5 minutes in the presence of 1 μ M CPI-818 (ITKi) or 1 μ M CPI-893 (ITK/RLKi). Auto-phosphorylation of ITK Y180 and PLC- γ Y783, an ITK substrate, were significantly reduced in the presence of either agent, confirming on target effects.
- (C) T8ML-1 cells (express ITK, but not RLK) were stimulated for 60 minutes with anti-CD3/CD28 beads in the presence of vehicle control, 1 μ M CPI-818 (ITKi), or 1 μ M CPI-893 (ITK/RLKi). Cells were lysed followed by nuclear and cytoplasmic fractionation. Western blot was performed on each fraction for p65. ITK inhibition with either agent resulted reduced NF- κ B nuclear translocation.
- (D) T8ML-1 cells (express ITK, but not RLK) were stimulated for 24 hours with anti-CD3/CD28 beads in the presence of vehicle control, 1 μ M CPI-818 (ITKi) or 1 μ M CPI-893 (ITK/RLKi). ITK inhibition with either agent significantly reduced GATA3 expression in these experiments.

Figure 2: CPI-818 has minimal effect on normal T cells



- (A) Peripheral blood T cells from healthy donors were isolated by negative selection. TCR stimulation with anti-CD3/CD28 beads for 24 hours increased viability by 359% in the presence of vehicle control when normalized to unstimulated cells (n=3). Treatment with 1 μ M CPI-893 (ITK/RLKi) reduced the cell viability to only 133% when compared to unstimulated controls (n=3, p = 0.0045). Treatment with 1 μ M CPI-818 (ITKi) had a numerically smaller, but still statistically significant effect (n=3, average viability 278%, p = 0.0056).
- (B) IL-10 concentration in cell culture supernatants was evaluated by ELISA after 24 hours with and without TCR stimulation (anti-CD3/CD28 beads - "B"). CPI-893 (ITK/RLKi), but not CPI-818 (ITKi) significantly reduced IL-10 production upon TCR stimulation (886 pg/mL vs 11.7 pg/mL, p < 0.0001)

Figure 3: CPI-818 inhibits malignant T cell growth & activation



- (A) T8ML-1 cells (express ITK, but not RLK) were incubated with or without TCR stimulating anti-CD3/CD28 beads ("B") in the presence of vehicle control DMSO, 1 μ M CPI-818 (ITKi), or 1 μ M CPI-893 (ITK/RLKi) with varying concentrations of vincristine for 24h. Both agents displayed single agent activity, but also sensitized the cells to the effects of vincristine. The IC50 for vincristine in unstimulated cells, TCR-stimulated cells, CP-818 (ITKi) treated stimulated cells, and CPI-893 (ITK/RLKi) treated stimulated cells was 0.049, 0.443, 0.066, and 0.091 nM, respectively (n=4 per group).
- (B) IL-10 production in culture supernatants after 24 hours was decreased when compared to TCR stimulated controls (15.97 pg/mL, n=3 for each group) with both CPI-818 (3.47 pg/mL, p < 0.0001) and CPI-893 (2.3 pg/mL, p < 0.0001)
- (C) Similar results to figure 3A were obtained in primary patient samples treated with romidepsin.

Conclusions

- CPI-818 is a potent ITK specific inhibitor, while CPI-893 inhibits both ITK and RLK
- Normal T cells express both ITK and RLK which can compensate for inhibition of ITK function
- Malignant T cells almost exclusively express ITK, or express RLK at very low levels
- CPI-818 impairs TCR signaling resulting in:
 - Impaired malignant T cell growth and proliferation
 - Increased chemosensitivity
 - Decreased GATA3 expression
- CPI-818 has minimal effect on normal T cells

Future Directions

- Collectively, our data support further preclinical and clinical studies with CP-818 in T-cell lymphoproliferative disorders.

References

- Wang, et al. Clin Cancer Res. 2017 May 15;23(10):2506-2515.
- Dubovsky, et al. Blood. 2013 Oct 10;122(15):2539-49.

Disclosures

JJB: Corvus Pharmaceuticals, Inc. (employment, equity ownership)
 JJJ: Corvus Pharmaceuticals, Inc. (employment, equity ownership)
 RAW: Incyte, Corp (research funding)