ITK Inhibitor Induces Dose-Dependent Th1 Skewing in Normal T Cells and is Active in Refractory T Cell Lymphomas

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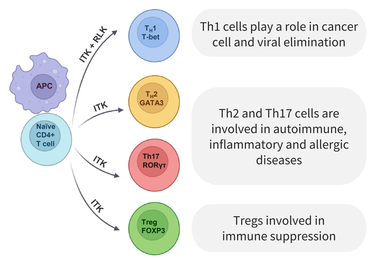
INTRODUCTION

Interleukin 2 inducible T cell kinase (ITK) is a TEC family tyrosine kinase expressed in T cells with an important role both in T cell receptor (TCR) signaling and T helper cell differentiation. Resting lymphocyte kinase (RLK), a closely related kinase, is redundant to ITK and is involved in the differentiation of naïve T cells into Th1 T cells. ITK-/- mice exhibit defects in Th2 differentiation while retaining the ability to differentiate into Th1 cells that secrete IFNγ. Th1 cells are involved in various cytotoxic T cell functions such as destruction of tumor cells; Th2 cells play a role in various inflammatory processes. CPI-818 is a covalent inhibitor of ITK (K_D 2.5nM) with > 100 fold selectivity over RLK and other TEC family kinases. CPI-818 is being evaluated in an ongoing phase 1 trial in patients (pts) with refractory T cell lymphomas (TCL). We now report that selective ITK blockade with CPI-818 induces: 1) Th1 skewing of normal T cells, 2) blockade of Th2 function and 3) anti-tumor activity.

CPI-818 IS SELECTIVE FOR ITK

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

ROLE OF ITK IN T CELL DIFFERENTIATION



Th1 cells play a role in cancer cell and viral elimination

Th2 and Th17 cells are involved in autoimmune, inflammatory and allergic diseases

Tregs involved in immune suppression

METHODS

IN VITRO AND PRECLINICAL STUDIES

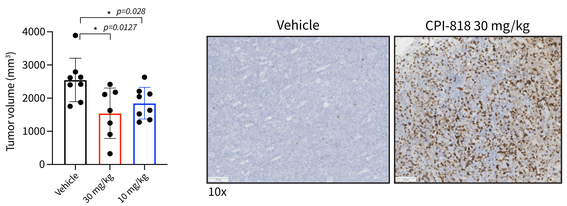
- Serum cytokines measured using MSD immunoassay format
- Lymphocyte subsets enumerated using flow cytometry on peripheral blood and tumor biopsy specimens using antibodies to CD3, 4, 8, 183, 196, 197 and 45RA
- C57BL/6 mice with established EL4 murine T cell lymphoma (CD4+/CD8-) were treated with CPI-818

PHASE 1 CLINICAL TRIAL IN REFRACTORY PERIPHERAL T CELL LYMPHOMAS

- Eligibility: failed all standard prior therapies
- Histologies included cutaneous T-cell lymphoma (CTCL), angioimmunoblastic T-cell lymphoma (AITL), peripheral T-cell lymphoma not otherwise specified (PTCL-NOS), anaplastic large-cell lymphoma (ALCL), and extranodal natural killer/T-cell lymphoma (ENKTCL)
- Design: successive cohorts of patients received CPI-818 100 mg, 200 mg, 400 mg, or 600 mg PO BID
- Endpoints: safety, immunologic activity and tumor response

IN VIVO TREATMENT OF MURINE T CELL LYMPHOMA

THERAPY INCREASES CD8+ INFILTRATION IN TUMORS

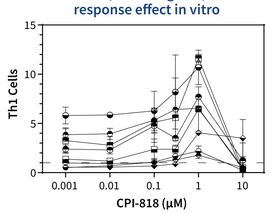


Mice with established EL4 tumors (CD4+) were treated with CPI-818 10, 30 mg/kg or vehicle daily for 7 days and tumor measurements/excision were made at Day 22 (3 days after dosing). Treatment groups were compared to control. Tumors were removed and immunohistochemistry was performed to enumerate infiltrating CD8+ normal T cells.

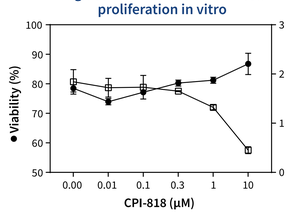
IN VITRO IMMUNOLOGY RESULTS

CPI-818 INDUCES TH1 SKEWING IN VITRO IN NORMAL PBMC

Th1 cells (ratio IFNg:IL-4) dose-response effect in vitro



High concentrations block T cell proliferation in vitro



Normal PBMC from 12 healthy donors were stimulated in vitro with anti-CD3/CD28 in the presence of various concentrations of CPI-818. Intracellular interferon gamma and IL-4 were measured using flow cytometry and Th1 skewing was determined by interferon gamma:IL-4 ratio. Effects on cell proliferation and viability were also determined. These results indicate that concentrations >0.01 and <10 μM induce Th1 skewing with anti-proliferative effects seen ≥1 μM.

IN VIVO PHARMACOKINETIC ANALYSIS

In vivo CPI-818 plasma concentration μM*

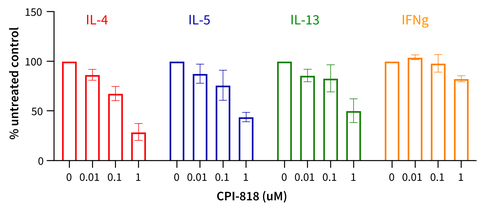
Dose (mg)	C_{max} (Std Dev)	C_{min} (Std Dev)
100 (N=4)	1.3 (1.0)	0.04 (0.1)
200 (N=5)	2.3 (1.9)	0.09 (0.2)
400 (N=5)	4.0 (2.3)	0.5 (1.1)
600 (N=11)	5.5 (4.7)	0.4 (0.6)

*geometric mean

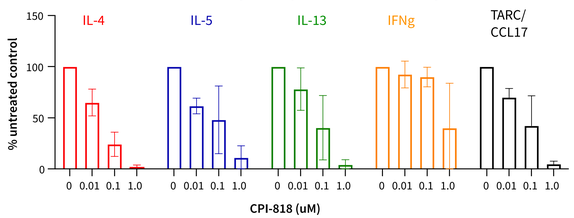
- PK studies indicate 200 mg dose provides optimum plasma concentration for Th1 skewing.
- Other studies on peripheral blood T cells showed that doses of 200 mg and higher provide maximal ITK target occupancy.

EFFECTS OF CPI-818 ON IN VITRO T CELL CYTOKINE PRODUCTION

CPI-818 inhibits Th2 cytokine production from normal donor CD4+ cells



CPI-818 inhibits Th2 cytokine production from Sezary cells



FACS-sorted CD4+ T cells from healthy controls (n=3; top panel) or from Sezary patients (n=2; bottom panel) were activated with anti-CD3/CD28/CD2-coated beads in the absence or presence of varying concentrations of CPI-818 for 72 hours. Supernatants were collected and Th2-associated cytokines, IFNg, and TARC (CCL17) were assessed by MSD.

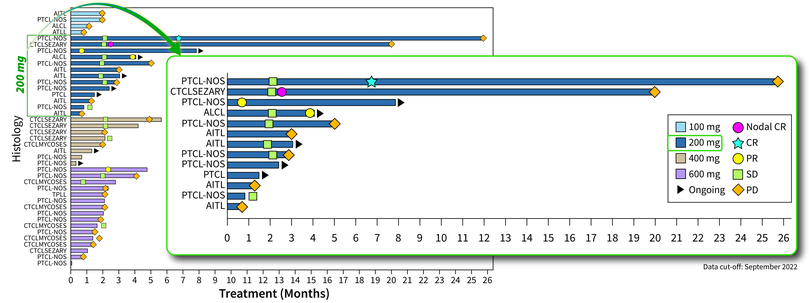
INTERIM PHASE 1 TRIAL RESULTS

PATIENT CHARACTERISTICS

	100 mg (N=4)	200 mg (N=13)	400 mg (N=10)	600 mg (N=16)
Age (yrs.), median (range)	51 (29, 75)	59 (29, 81)	66.5 (36, 80)	63.5 (34, 84)
Gender, male N (%)	3 (75)	4 (31)	6 (60)	8 (50)
No. of prior therapies, median (range)	3.5 (2, 4)	3 (1, 6)	4 (2, 15)	5 (1, 9)
Histologies				
PTCL-NOS	1	7	3	9
AITL	1	4	2	0
ATLL	1	0	0	0
ALCL	1	1	0	0
T-PLL	0	0	0	1
CTCL Sézary syndrome	0	1	4	1
CTCL Mycosis fungoides	0	0	1	5

ANTI-TUMOR ACTIVITY IN VARIOUS DOSING COHORTS

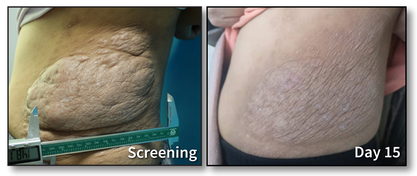
200 mg BID identified as optimal dose with ORR of 4/11 evaluable



ADVERSE EVENT SUMMARY

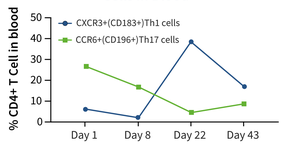
- The most common (≥ 10%, all causality) AEs were nausea, chills, vomiting, fatigue, pyrexia, decreased appetite, pruritus, and rash.
  - Treatment-related Grade 3+ AEs were: anemia (1), lymphocytosis (1), WBC decreased (1) and neutropenia (1).

ANTI-TUMOR ACTIVITY

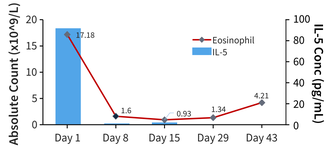


- PTCL-NOS patient failed CHOEP, GDP, HDACi, and anti-PD1.
- Large subcutaneous mass on abdomen.
- Has PR 8+ months duration in all sites of disease (bone marrow, skin, lymph node, and spleen) on CPI-818.

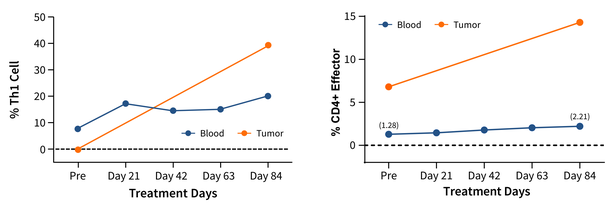
Frequency of Th1 & Th17 CD4 Cells in Blood



Effects on Eosinophils and Serum IL-5



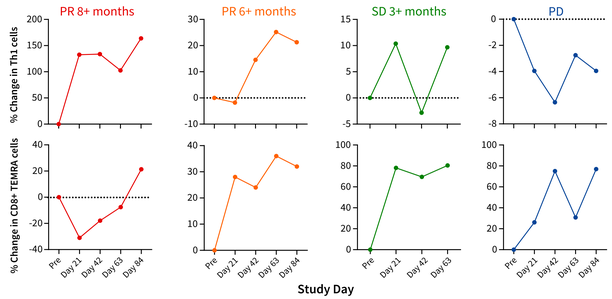
Th1 and CD4+ Effector Cells Increase in Blood and Tumor During CPI-818 Treatment



PBMC and tumor samples were analyzed at various timepoints and demonstrate increase in blood Th1, decrease in blood Th17, reduction of both eosinophil count and IL-5 consistent with Th1 skewing and Th2 blockade. Baseline and on treatment tumor biopsy and blood show increase in Th1 and terminally differentiated T effector memory cells.

EFFECTS OF CPI-818 ON DIFFERENTIATION OF TH1 AND CD8+ TEMRA CELLS

Percent Change in Th1 and CD8+ TEMRA Cells from Baseline



Peripheral blood from four patients were collected at various timepoints and T cell subsets evaluated by flow cytometry. Plots show percent change from baseline for Th1 (CD3+/CD4+/CD8-/CD183+/CD196-) and for CD8+ TEMRA cells (CD3+/CD4-/CD8+/CD197-/CD45RA+). Two PR patients and SD patient show increases in Th1 and TEMRA cells while on treatment with CPI-818. Of note, the SD and PD patients were lymphopenic at baseline (<1000 ALC).

CONCLUSIONS

- **CPI-818 is a selective ITK inhibitor that at optimal concentrations induces Th1 skewing and blockade of Th2 function and related cytokine production (e.g., IL-4, IL-5, IL-13, TARC).**
- **In an ongoing international Phase 1 trial:**
  - **Durable objective responses seen (1 CR, 25 mo; 1 nodal CR, 19 mo; 2 PRs, 6+, 8+ mo) in 11 evaluable patients at optimum dose.**
  - **Doses up to 600 mg po BID have been well-tolerated with no dose limiting toxicities.**
  - **Baseline and on-treatment blood and tumor biopsy specimens show increases in both Th1 cells (CD3+/CD4+/CD8-/CD183+/CD196-) and in terminally differentiated T effector memory cells (TEMRA: CD3+/CD4-/CD8+/CD197-/CD45RA+).**
- **Increases in Th1 cells and TEMRA CD8+ cells in humans and preclincal data suggest that anti-tumor activity is due to induction of a host anti-tumor response.**
- **Selective ITK inhibition may have the potential to enhance anti-tumor immunity and also could be useful in the therapy of Th2-mediated autoimmune/allergic diseases.**
- **A Phase 2 trial in refractory peripheral T cell lymphomas is planned.**