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Biochemical, Immunologic and In Vivo Preclinical Studies with CPI-818: A Selective Interleukin-2-Inducible T-cell Kinase Inhibitor That Inhibits T-Cell Receptor Signaling, Promotes Th1 Skewing, and is Efficacious in Dogs with T-Cell Lymphomas

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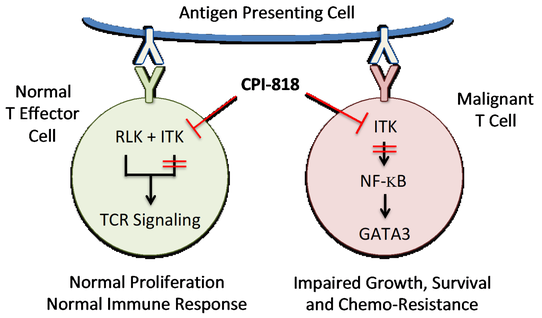
ABSTRACT

Background: ITK is a non-receptor tyrosine kinase that modulates TCR signaling (August *et al.* 2012). In T-cell lymphoproliferative disorders, expression of the TCR and its downstream signaling components are maintained suggesting malignant T cells may exploit this pathway for growth and survival. Professional antigen presenting cells (APCs), abundant in the lymphoma microenvironment, may provide antigen to drive TCR signaling through ITK, which is consistently expressed in a variety of T-cell lymphomas including peripheral T-cell lymphoma (PTCL), cutaneous T-cell lymphoma (CTCL) and angio-immunoblastic T-cell lymphoma (Agostinelli *et al.* 2014). In addition, activation of ITK in malignant T cells has been shown to up regulate GATA-3, a transcription factor that drives Th2 differentiation and that is overexpressed in PTCL with poor overall survival (Wang *et al.* 2014, Iqbal, *et al.* 2014, Wilcox *et al*, 2016) suggesting that selective ITK inhibition may be particularly beneficial to these patients. Selective pharmacologic inhibition of ITK, sparing RLK, may be necessary to inhibit Th2 responses without affecting Th1 dependent immunity. We report the discovery and characterization of CPI-818, an irreversible inhibitor of ITK. CPI-818 possesses a high degree of selectivity for ITK over RLK allowing for assessment of the impact of selective ITK inhibition on normal and malignant T-cells.

Results: CPI-818 irreversibly inhibited ITK (IC_{50} 2.3 nM) with >100-fold selectivity over RLK (430 nM) and BTK (850 nM). The mechanism of ITK inhibition involves covalent binding to CYS-442 confirmed by mass spectrometry. Irreversible inhibition of ITK *in vitro* and *in vivo* was demonstrated using an active site competitive probe. In cellular assays, CPI-818 inhibited anti-CD3/28 induced phosphorylation of ERK (T202/Y204) and PLCγ (Y783) in PMBCs, and inhibited IL-2 secretion in Jurkat cells (IC_{50} 76 nM). CPI-818 demonstrated dose-dependent inhibition of TCR-induced proliferation of malignant T-cells from Sézary syndrome patients. In mice orally treated with CPI-818 an increase in the ratio of IFNγ/IL-4 ($p<0.05$) upon antigen-specific re-stimulation in an OVA transgenic adoptive transfer model was observed, consistent with Th1-skewing.

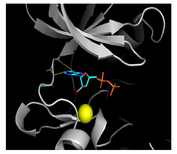
To assess the potential of CPI-818 to treat human T-cell lymphoproliferative disorders, the safety and efficacy of CPI-818 in companion dogs that spontaneously developed T-cell lymphomas was evaluated. CPI-818 was given orally at a dose of 20 mg/kg BID for durations ranging from 2 weeks to 5 months. Three animals were treated: one with PTCL and two with CTCL. Full ITK occupancy in peripheral blood was confirmed using the probe assay. Evidence of anti-tumor activity was seen in all dogs including complete and partial responses. CPI-818 was well tolerated with no change in normal lymphocyte counts. These data support evaluation of CPI-818 in clinical trials in patients with T-cell malignancies.

INTRODUCTION

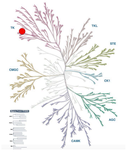


RESULTS

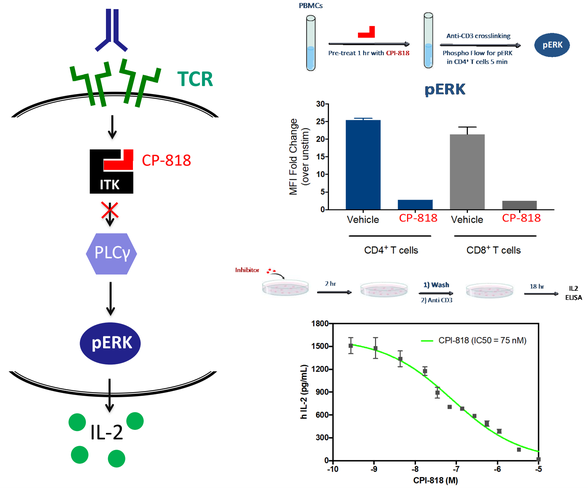
CPI-818 is a potent ITK inhibitor and selective versus the "cysteinome"



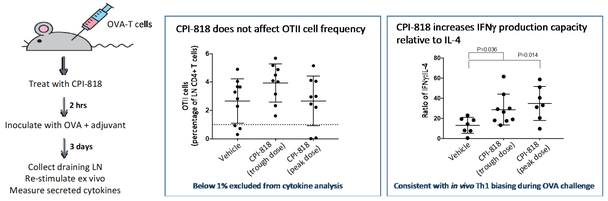
Kinases of the "Cysteinome"	Active Site Sequence of Kinase	CPI-818 (Kd in nM)
ITK	APICLVFEFMEHGCLSDYLRTGRGL	6.5
TEC	KPIYIVTEFMEHGCLLNFLRQRQGH	540
BTK	RPIFITTEYMANGCLLNYLREMRHR	1,200
RLK	KPLYIVTEFMENGCLLNYLRENKGK	2,700
JAK3	PELRLVHKYLPSGCLRDFLQRHRAR	2,800
BLK	EPIYIVTEYMARGCLLDFLKTDEGS	4,700
BMX	YPLYLVTEYLSNGCLLNYIRSHGKG	9,100
EGFR	STVQLITQLMPFGCLLDYVREHKDN	>10,000
ERBB2	STVQLVTQLMPYGCLLDHVRENRGR	>10,000
ERBB4	PTIQLVTQLMPHGCLLEYVHEHKDN	>10,000



Inhibition of signal transduction and cytokine production by CPI-818



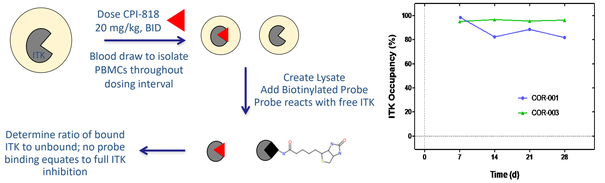
CPI-818 promotes IFNγ production (Th1 biasing)



Splenocytes (2.5M cells) from CD45.2+ OVA-specific OTII mice were adoptively transferred to CD45.1+ recipient mice. The following day mice were immunized with OVA protein (50 µg) plus AddaVax adjuvant (50%) and oral administration of CPI-818 (50 mg/kg) BID was initiated. Three days later, draining lymph nodes were collected either prior to CPI-818 dosing (trough dose) or 2 hours post-dose (peak dose) for re-stimulation with OVA peptide and assessment of secreted IFNγ and IL-4. Data are representative of two independent experiments.

RESULTS (Cont)

Pharmacodynamic assessment of ITK inhibition by CPI-818 in vivo



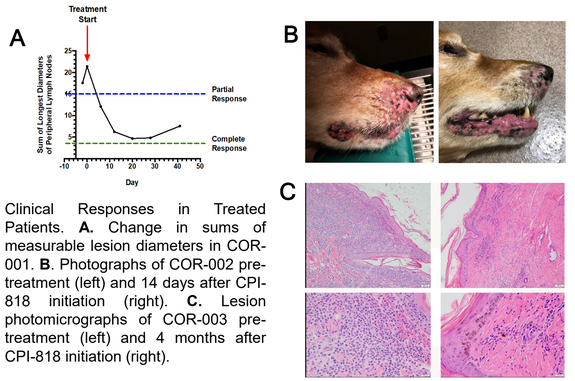
Bioanalysis to determine plasma concentrations of CPI-818 was performed using standard liquid chromatography–MS/MS methods. The active site occupancy of ITK by CPI-818 was measured using an irreversible, biotinylated probe in peripheral blood mononuclear cells (PBMCs) obtained from animals dosed with CPI-818. ITK occupancy was measured at trough concentrations at week 1, 2, 3 and 4 for two patients (COR-001 and COR-003).

Baseline characteristics of companion animals with PTCL and CTCL

Study ID	Signalment	Weight (kg)	Administered Dose (mg/kg)	Lymphoma Subtype	Best Response	Time On Study
COR-001	7 yo FS Boxer	26	19.2	PTCL-NOS	PR	41 d
COR-002	11 yo MC Golden Retriever	35.2	19.9	CTCL (mucocutaneous)	MR	16 d*
COR-003	11 yo FS Golden Retriever	34.1	20.5	CTCL - LN involvement	CR	160 d

FS: Female spayed. MC: male castrated. PTCL-NOS: Peripheral T cell lymphoma – not otherwise specified. CTCL: Cutaneous T cell lymphoma. LN: Lymph node. PR: Partial response. MR: Minor response. CR: Complete response.
* Removed from study for unrelated neoplasm

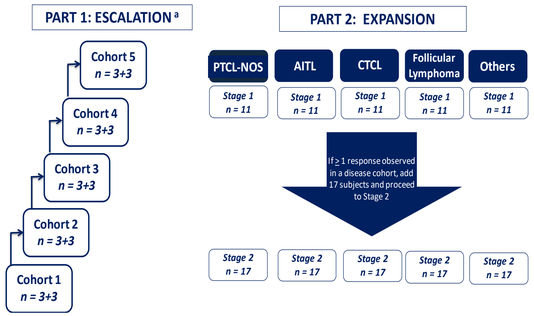
Inclusion Criteria
Age: At least one year old.
Body weight: At least 10 kg.
Histologic or cytologic diagnosis of lymphoma confirmed. Documentation of T cell immunophenotype by means of CD3 or CD5 immunoreactivity through immunohistochemistry, immunocytochemistry or flow cytometry, or monoclonal T cell receptor gene rearrangement by PCR for antigen receptor rearrangement.
Adequate organ function as indicated by standard laboratory tests: (hematology (CBC), clinical chemistry and urinalysis). Performance status of either 0 or 1 on Day 0, according to modified ECOG Performance Scheme (Veterinary and Comparative Oncology, 2016).
CSU IACUC/Clinical Review Board approval; Written, informed consent from owner.



Clinical Responses in Treated Patients. **A.** Change in sums of measurable lesion diameters in COR-001. **B**. Photographs of COR-002 pre-treatment (left) and 14 days after CPI-818 initiation (right). **C.** Lesion photomicrographs of COR-003 pre-treatment (left) and 4 months after CPI-818 initiation (right).

CLINICAL PLAN

Plan for phase 1/1b clinical trial for CPI-818 in humans



CONCLUSIONS

- CPI-818 is a selective, covalent inhibitor of ITK (sparing RLK and BTK)
- CPI-818 blocks signal transduction in proximal (pERK) and distal endpoints (IL-2 secretion) downstream of T-cell activation
- Through selective ITK inhibition, CPI-818 promotes IFNγ production in an OTII adoptive T-cell transfer model
- BID dosing of CPI-818 at 20 mg/kg in dogs provides durable ITK inhibition
- In a companion animal study in dogs with PTCL and CTCL, CPI-818 was well tolerated with evidence for clinical responses
- A FIH study of CPI-818 is planned in FL, AITL, PTCL-NOS and CTCL

REFERENCES and FOOTNOTES

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