CPI-818: A SELECTIVE INTERLEUKIN-2-INDUCIBLE T-CELL KINASE INHIBITOR HAS CLINICAL ACTIVITY IN DOGS WITH SPONTANEOUS T CELL LYMPHOMA

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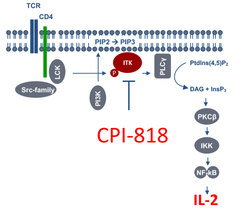
ABSTRACT

Background: ITK is a non-receptor tyrosine kinase that plays a key role in T cell receptor (TCR) signaling. In malignant T cells, TCR signaling is maintained, and inhibition of ITK may provide therapeutic benefit. Non-Hodgkin lymphoma (NHL), the most common hematopoietic tumor of dogs, is an aggressive and highly metastatic disease. Approximately 30% of lymphomas are of the T cell immunophenotype, and most correlate closely with human intermediate-to high-grade NHL, with peripheral T cell lymphoma being the most common histotype. CPI-818 is an irreversible small molecule inhibitor of ITK that possesses a high degree of selectivity for ITK over RLK or Bruton's tyrosine kinase (BTK), allowing for assessment of the impact of selective ITK inhibition on malignant T cells. Given that ITK activation may require coordinated signaling through the TCR, conventional murine xenografts may not accurately recapitulate the microenvironment necessary for modeling the effect of ITK inhibition on lymphoma progression. For this reason, clinical, pharmacokinetic and pharmacodynamic evaluation of CP-818 was performed in dogs with spontaneous T-cell lymphoma.

Materials and Methods: To assess the potential of CPI-818 to treat T cell lymphoproliferative disorders, the safety and efficacy of CPI-818 in client-owned dogs with spontaneously-occurring T cell lymphoma was evaluated. This trial was performed with Institutional Animal Care and Use Committee approval and signed owner consent. CPI-818 was given orally at a dose of 20 mg/kg BID for from 2 weeks to 5 months. Owner history, physical examination and clinicopathologic evaluation was performed weekly for the first 4 weeks, then every other week thereafter. Irreversible inhibition of ITK in peripheral blood lymphocytes was measured using a labeled competitive probe to assess ITK occupancy. Tumor biopsies were obtained prior to treatment, 2 weeks following treatment initiation and at the time of relapse, and Affy-based gene expression profiling and pathway analysis was performed.

Results: Three animals have been treated to date: 1 with peripheral T cell lymphoma (PTCL) and 2 with cutaneous T cell lymphoma (CTCL). Full ITK occupancy in peripheral blood was confirmed using the probe assay in all 3 dogs. Evidence of anti-tumor activity was observed in all dogs including complete and partial responses. CPI-818 was well tolerated with no change in normal lymphocyte counts. Gene expression pathways significantly upregulated following CP-818 exposure included those associated with cytokine signaling and interferon response.

Conclusions: CPI-818 is a novel and selective ITK inhibitor that blocks TCR-driven signaling. Evidence of *in vivo* ITK inhibition and clinical anti-tumor activity with excellent tolerability was shown in client-owned dogs with spontaneous T cell lymphoma.



METHODS

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.

Exclusion Criteria:

- Dogs of B cell or null-cell immunophenotype.
- Dogs that have received chemotherapy within 3 weeks of Day 0.
- Dogs that have received radiation therapy within 6 weeks of Day 0.
- Dogs that have received corticosteroids within 72 hours of Day 0.
- Concurrent malignancy, other than lymphoma, or other serious systemic disorder incompatible with the study.
- Dogs on alternative/complementary therapies.

METHODS (continued)

Treatment and Monitoring:

- CPI-818 was administered orally twice daily at 20 mg/kg.
- *Assessments:* owner history, physical examination, lesion measurements, complete blood count, serum biochemistry profile, coagulation analysis and urinalysis. Performed weekly for the first 4 weeks, then every other week thereafter.
- After 1 week of CPI-818 dosing, plasma was serially collected for 10-point pharmacokinetic analysis. Peripheral blood mononuclear cells (PBMC) collected prior to treatment initiation, Week 1 pre-dose, 4 and 24 hours post-dose for assessment of ITK drug occupancy.

Response and Adverse Effect Assessment:

- Responses were measured using the Veterinary Cooperative Oncology Group (VCOG) Response Criteria for Peripheral Nodal Lymphoma v1.0. (Vail et al, 2009) or the VCOG RECIST criteria v1.0 (Nguyen et al, 2013).
- All adverse events were graded according to the Veterinary Comparative Oncology Group Common Terminology criteria for Adverse Events (VCOG-CTCAE) v1.1 (Veterinary and Comparative Oncology, 2016).

Pharmacokinetic and Pharmacodynamic Analysis:

- 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. Binding of CPI-818 and the probe to the active site is mutually exclusive (**Figure 1**). In lysates of PBMCs collected from treated animals, the biotinylated probe reacts with unoccupied ITK allowing for the determination of the extent to which ITK is inhibited by CP-818. ITK occupancy was measured at trough concentrations at week 1, 2, 3 and 4 for two patients (COR-001 and COR-003).

Gene Expression Analysis

- RNA was extracted from lymphoma biopsies prior to treatment and after 2 weeks of CPI-818 treatment in COR-001, and QC'd through NanoDrop and Agilent BioAnalyzer.
- Labeled and hybridized to Affymetrix Canine Genome 2.0 chips and scanned
- Data normalized across chips using RMA. Normalized expression data were called with SimpleAffy and expression values registered using Limma.
- Transcripts with Log2 fold-change >2 utilized for pathway analysis via Reactome software (www.reactome.org)

Table 1. *Patient Information*

Study ID#	Signalment	Body 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

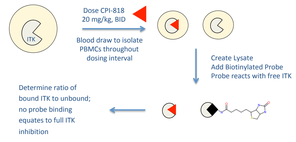


Figure 1. *Pharmacodynamic assessment of ITK Inhibition in PBMCs following oral CPI-818 dosing*

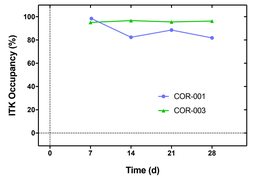


Figure 2. *ITK occupancy measured in PBMCs from patient dogs while on study (first month)*

Table 2. *Reported Adverse Effects*

	Adverse Event Type	Highest Grade	
COR-001	Hyporexia	2	Attributed to stress
	Vomiting	2	
	Diarrhea	2	
	Polydipsia	1	
	Biopsy site swelling	1	
	Lymphopenia	1	
	Hyperproteinemia	1	
	Adverse Event Type	**Highest Grade**	
COR-002	Increased BUN	1	Attributed to concurrent neoplasia (hemangiosarcoma)
	Hypochloremia	1	
	Hyperproteinemia	1	
	Thrombocytopenia	1	
	Anemia	1	
	Pelvic limb weakness	1	
	Pericardial effusion	3	
	Adverse Event Type	**Highest Grade**	
COR-003	Vomiting	1	Bacterial cholangiohepatitis and secondary aspiration pneumonia
	Fever	2	
	Tachypnea	1	
	Thrombocytopenia	3	
	Peripheral edema	1	
	Hyperbilirubinemia	2	
	ALT/AST elevation	3	
	Hypoglycemia	1	
	Lethargy	2	

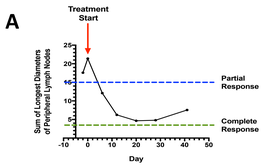


Figure 3. *Clinical Responses in Treated Patients*. **A.** Change in sums of measurable lesion diameters in COR-001. **B.** Photographs of COR-002 pre-treatment (left) and 14 days after CPI-818 initiation (right). **C.** Lesion photomicrographs of COR-003 pre-treatment (left) and 4 months after CPI-818 initiation (right).

Table 3. *Differentially Represented Gene Expression Pathways in Lymphoma Tissue Following CPI-818 Treatment (reactome.org)*

	Entities Found	Entities Total	Entities P value	Entities FDR
Cytokine Signaling in Immune System	32	1,051	1.11E-16	1.99E-14
Interleukin-10 signaling	12	86	5.22E-15	4.64E-13
Immune System	39	2,638	4.17E-14	2.46E-12
Interferon alpha/beta signaling	14	184	8.37E-14	3.68E-12
Interferon Signaling	15	388	1.35E-10	4.72E-09
Signaling by Interleukins	16	640	1.51E-08	4.38E-07
Chemokine receptors bind chemokines	5	48	2.73E-06	6.82E-05
Interleukin-4 and Interleukin-13 signaling	8	211	4.67E-06	1.03E-04
Antiviral mechanism by IFN-stimulated genes	5	83	3.74E-05	6.36E-04
ISG15 antiviral mechanism	5	83	3.74E-05	6.36E-04

CONCLUSIONS

- A dose of 20 mg/kg CPI-818 BID is associated with plasma exposures predicted to be associated with full occupancy of ITK.
- High ITK occupancy at both peak and trough plasma concentrations was confirmed in peripheral blood of dogs with T cell lymphoma following 1 week of CPI-818 dosing.
- CPI-818 tolerability was excellent in dogs with spontaneous T cell lymphoma.
- Objective evidence of antitumor activity was documented in 3 of 3 dogs treated with CPI-818.
- Gene expression profiling in lymphoma tissue pre- and post-treatment identified modulation in pathways associated with cytokine/chemokine signaling and interferon response.

ACKNOWLEDGEMENTS/DISCLOSURES

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