PRECLINICAL CHARACTERIZATION OF PRN1008, A NOVEL REVERSIBLE COVALENT INHIBITOR OF BTK THAT SHOWS EFFICACY IN A RAT MODEL OF COLLAGEN-INDUCED ARTHRITIS

#THU0068

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Introduction

Bruton's Tyrosine Kinase (BTK) is a cytoplasmic signaling molecule downstream from a group of cellular receptors important for disease initiation, propagation, and tissue destruction associated with a variety of autoimmune diseases including rheumatoid arthritis. There is strong pre-clinical validation for BTK as a therapeutic target for autoimmune diseases based on multiple animal models. Principia discovered a potent, selective inhibitor of BTK that targets cysteine through a reversible covalent interaction which results in prolonged residence time and durable inhibition of the target.

Methods

Biochemical characterization of PRN1008 was performed utilizing Caliper-based kinase assays, TR-FRET-based off-rate assays, and mass-spectrometry-based reversibility assays. Binding of PRN1008 to BTK was assessed in Ramos B cells, human PBMC and rat splenocytes (for PK/PD studies) using a fluorescent probe-based occupancy assay. Impacts of PRN1008 on B cell function were assessed by B cell CD69 expression in human whole blood (HWB) and proliferation of purified human primary B cells induced by anti-IgM. Cellular selectivity for BTK was demonstrated by lack of potency against a range of off-target cell-based assays. The in vivo efficacy of PRN1008 was tested in a rat model of collagen-induced arthritis.

Results

PRN1008 was found to be very potent against BTK (IC50 = 1.3 ± 0.5 nM) and highly selective when tested against a panel of 251 other kinases. Cysteine targeting of BTK by PRN1008 results in a slow off-rate demonstrated by retention of 79 ± 2% of binding to BTK in PBMC 18 hours after washing away the compound in vitro. The covalent cysteine binding was completely reversible after denaturation of the target. Anti-IgM induced human B cell proliferation (10% serum) and B cell CD69 expression (whole blood) were inhibited by PRN1008 with IC50 of 5 ± 2.4 nM and 123 ± 38 nM, respectively. PRN1008 did not block EGFR signaling in epithelial cells or TCR and calcium flux stimulated T cell activation. PRN1008 also did not block IL-4 stimulation of B cells and did not exhibit cytotoxicity in an epithelial cell line HCT-116. In addition, PRN1008 did not block antibody dependent cell-mediated cytotoxicity in combination with anti-CD20 antibodies allowing for potential combination therapies. In vivo PRN1008 demonstrated enduring pharmacodynamic effects after the compound had cleared from circulation, consistent with extended target residence time. PRN1008 also reversed and completely suppressed collagen-induced arthritis in rats in a dose dependent manner which allowed correlation of target occupancy and disease modification.

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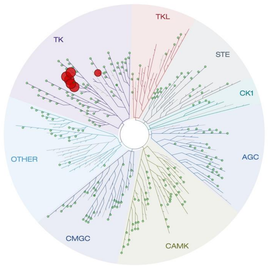
In Vitro Characterization of PRN1008

Biochemical and cellular screening of PRN1008 shows high selectivity and potency



6 of 250 kinases >90% inhib @ 1 µM

Conserved Cysteine Panel[1]			
Kinase	IC_{50} (nM)	Kinase	IC_{50} (nM)
BTK	1.3 ± 0.5	BLK	6.3 ± 0.7
BMX	1.0 ± 0.1	EGFR	520 ± 170
ITK	440 ± 100	ERBB2	3900 ± 940
TEC	0.8 ± 0.1	ERBB4	11.3 ± 6.5
RLK	1.2 ± 0.3	JAK3	>5000

[1]Kinases that contain a Cys homologous to Cys-481 in BTK

Cellular efficacy assays	
B cell activation in HWB (IC_{50}) [1]	123 ± 38 nM
Occupancy of BTK in PBMC (HWB) (IC_{50}) [2]	233 ± 75 nM
Basophil activation in HWB (IC_{50}) [3]	490 ± 130 nM
Human primary B cell proliferation (IC_{50}) [4]	5 ± 2.4 nM
Human Ramos B cell occupancy (IC_{50}) [2]	8 ± 2 nM

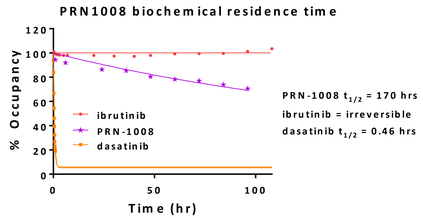
[1]Anti-IgM-induced CD69 expression on CD20+ cells after 18h following 1h incubation with cmpd.
[2]Occupancy was measured by binding of an irreversible fluorescent probe to unbound BTK.
[3]Anti-IgE-induced degranulation of basophils by CD63 expression.
[4]Anti-IgM-induced proliferation of purified human B cells after 48 h in culture.

Cellular cross-screen selectivity assays	
TCR-induced reporter assay in Jurkat cells (IC_{50})[1]	> 5000 nM
Ca flux-induced reporter assay in Jurkat cells (IC_{50}) [1]	> 5000 nM
IL-4 – induced STAT6 reporter assay in Ramos cells (IC_{50}) [1]	> 5000 nM
EGFR reporter assay in ME-180 cells (IC_{50}) [1]	> 5000 nM
Cytotoxicity in HCT-116 cells (IC_{50}) [2]	> 5000 nM

[1]Invitrogen CellSensor® reporter assays based on beta-lactamase activation.
[2]Cell viability measured after 72 h in culture by Cell-titre Glo assay (Promega).

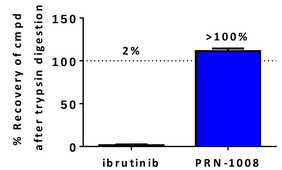
Durability of BTK binding in cells	
Occupancy of BTK in PBMC 4h, 18h post washout	91%, 79%

Reversible covalent BTK inhibitor PRN1008 demonstrates slow off-rate kinetics



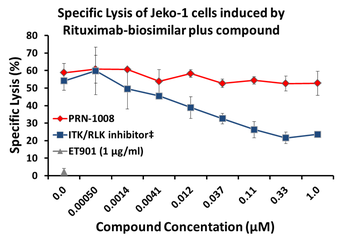
Off-rate was measured by TR-FRET detection of high affinity tracer binding following compound binding to BTK and subsequent dilution to allow dissociation to occur.

Reversibility of binding is demonstrated by complete recovery of PRN1008 after digestion with trypsin



Recovery of free compound was measured by mass spectrometry following binding to excess BTK followed by digestion with trypsin.

PRN1008 does not block ADCC, demonstrating selectivity and lack of interference with anti-B cell antibodies



Antibody-dependent cell-mediated cytotoxicity was measured by lysis of Jeko-1 B cells pre-coated with ET901 anti-CD20 antibody and incubated with PBMC (E/T 25:1) detected by LDH release assay.
‡Principia ITK/RLK inhibitor shown to inhibit ADCC (Zhong et al., JBC 290:5960, 2015).

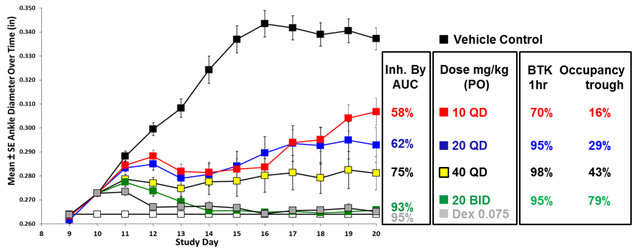
In Vivo Efficacy of PRN1008

BTK occupancy is maintained after PRN1008 has cleared from the plasma

PO-PK/PD*	1hr	4hr	12hr	24hr
Occupancy	95 %	91 %	80 %	29 %
Plasma PK (ng/ml)	128 ng/ml	106 ng/ml	4.6 ng/ml	<LLOQ[1]

*** 20 mg/kg q.d. dose; [1]Lower limit of quantification.**

In vivo efficacy in rat collagen-induced arthritis model achieved after oral dosing with less than maximal trough occupancy



	Inh. By AUC	Dose mg/kg (PO)	BTK 1hr	Occupancy trough
	58%	10 QD	70%	16%
	62%	20 QD	95%	29%
	75%	40 QD	98%	43%
	93% 95%	20 BID	95%	79%
		Dex 0.075		

Joint damage prevented by treatment with PRN1008

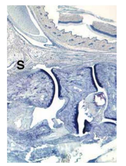
Micro CT scans

Vehicle Control.
Severe pitting and joint damage

20 mg/kg bid.
No joint damage

Histopathology images

Vehicle Control.
Severe inflammation and cartilage damage (arrows); mild periosteal bone formation (P)



20 mg/kg bid.
Ankle from treated animal is essentially normal

Conclusions

PRN1008 is a potent, selective and reversible covalent inhibitor of BTK with extended PD effects in vivo and efficacy in collagen-induced arthritis in rats. These efficacy data, together with the PK, PD, target occupancy, and downstream biomarker data achieved in our Phase 1 clinical trial, suggest the potential for success in treatment of RA.

(See SAT0232 for Ph1 human data).