Phase 1 Clinical Trial of PRN2246 (SAR442168), a Covalent BTK Inhibitor Demonstrates Safety,CNS Exposure and Therapeutic Levels of BTK Occupancy

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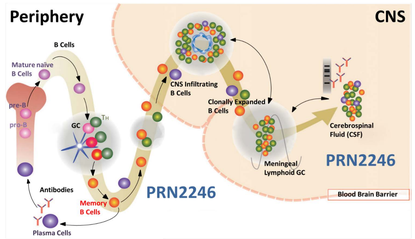
Introduction

The role of B cells in the pathophysiology of multiple sclerosis (MS) has been validated by the clinical results of B lymphocyte depletion with ocrelizumab, an anti-CD20 antibody.[1] Bruton's tyrosine kinase (BTK), expressed in B cells, innate immune cells and microglia, is an essential signaling element downstream of the B-cell receptor and Fc-receptors. PRN2246 is a potent, brain penetrant BTK inhibitor that covalently binds BTK, resulting in prolonged inhibition with the potential to target inflammation in the periphery and central nervous system. PRN2246 demonstrates durable (>24 hours) BTK occupancy in biochemical assays which translates into long duration of action in cellular systems. *In vitro,* PRN2246 can inhibit BTK dependent inflammatory immune mechanisms in multiple immune cell types. Of relevance to MS, PRN2246 potently inhibits BCR signaling to down regulate B cell activation and maturation, and can inhibit BTK in microglial cells isolated from the central nervous system.

Figure A[2]. PRN2246 has potential to act in CNS and periphery

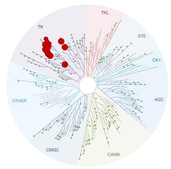


Non-clinical Pharmacology

PRN2246 demonstrates potent binding in Ramos and microglia-HMC cell lines and potent activity in a HWB assays. In a screen across 250 kinases 12 of 250 kinases exhibited >90% inhibition at 1μM (Figure B).

Figure B. PRN2246 activity and selectivity

On-target cell based IC50		(nM)
BTK	Ramos cells[1]	0.4
BTK	Microglia- HMC cells[1]	0.7
BCR	HWB- B cells[2]	10



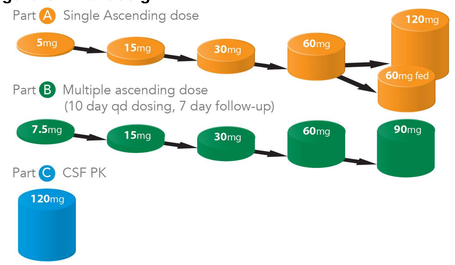
Left panel. All cell types treated with PRN2246 for 1hr at 37°C then:
[1]BTK occupancy measured by binding of an irreversible fluorescent probe in Ramos B cell line (ATCC) or Microglia (HMC) cell line
[2]Anti-IgM-induced CD69 expression on CD20+ cells in HWB after 18h stimulation measuring B Cell Receptor (BCR) stimulation
Right panel. Kinase selectivity profile of PRN2246 screened against 250 kinases. Dots represent individual kinase with > 90% inhibition at 1 μM.

In vivo occupancy of BTK was demonstrated in preclinical studies in both mice and rats. PRN2246 demonstrated dose-dependent protection from disease induction in a mouse model of EAE, a neuroinflammatory model with similarities to human MS, with durable BTK target occupancy confirmed for up to 24 hours in both the periphery and CNS of treated mice.[3]

Trial Design

This first-in-human randomized, double-blind, placebo-controlled study was comprised of 5 SAD arms, 5 MAD arms with 10 days treatment, and one arm in which CSF exposure was measured 2 hr after a single dose. At one dose (60mg), following a washout of 1 week, subjects returned and were administered PRN2246 after a moderate fat meal. In Part C, 4 subjects were administered PRN2246 under fasted conditions and CSF exposure was assessed from a lumbar puncture at 2h. Peripheral BTK occupancy was assessed at various timepoints by an ELISA based readout using an irreversible probe.

Figure C. Trial design



PK Results

PRN2246 was rapidly absorbed following oral administration, with a median Tmax of ~1 hour. The plasma half-life was approximately 2h, and increases in exposure were approximately dose-proportional from 15 mg to 60 mg. Less than 1% of PRN2246 was excreted unchanged in urine, and food increased exposure. Modest accumulation was observed with multiple dosing.

Figure D. PRN2246 exposure.

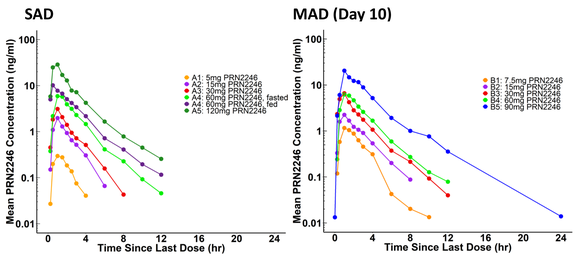


Table A. Multiple Dose PRN2246 Pharmacokinetics (Day 10)

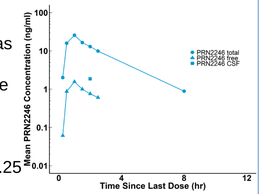
Dose (n)	Tmax (h)	Cmax (ng/mL)	AUC24 (ng.h/mL)	T1/2 (h)
7.5 mg QD (8)	1.0 (0.5-2.0)	1.3 (31)	3.1 (51)	1.6 (50)
15 mg QD (8)	1.0 (0.5-1.5)	2.5 (60)	5.6 (33)	1.7 (21)
30 mg QD (8)	1.0 (0.5-2.0)	7.5 (74)	14.9 (75)	2.0 (34)
60 mg QD (7)	1.0 (1.0-2.0)	6.7 (66)	18.2 (55)	2.4 (44)
90 mg QD (8)	1.0 (1.0-2.5)	21.6 (59)	56.6 (54)	2.8 (30)

Data reported as mean (CV%); Tmax median (range)

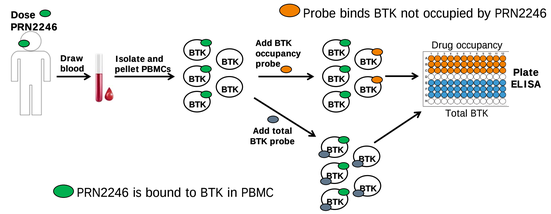
CSF Exposure

Figure E. CNS exposure of PRN2246 (Part C)

CSF exposure was assessed via lumbar puncture at 2h in 4 subjects. PRN2246 was ~93.5% bound to plasma proteins and exhibited CSF penetration 2h after a single 120 mg dose. The Gmean CSF concentration was 1.87 ng/mL, which exceeds the cell based in vitro IC_{90}. The CSF:plasma ratio of free PRN2246 was 2.25



Assessment of BTK Occupancy



- BTK Occupancy at Day 1 increases in a dose dependent manner with full occupancy at doses >60mg
- Occupancy increases with time with all doses approaching full occupancy by Day 10
- Free BTK levels return toward normal over 7 days due to protein resynthesis

Figure G. BTK occupancy of PRN2246.
Levels of BTK occupancy were measured at 4, 12 and 24 hours after dosing on Day 1, pre-dose on days 3, 7, and 10, and at multiple timepoints out to 168h (7 days) after administration of the final dose. All occupancy measurements were normalized to predose levels from Day 1.

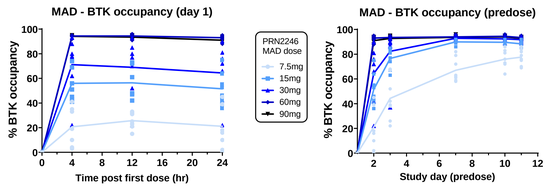


Table B. PRN2246 BTK occupancy (MAD)

Dose (h post dose)	Placebo*	7.5mg	15mg	30mg	60mg	90mg
D1 (4)	4 ± 5	21 ± 16	56 ± 13	71 ± 22	94 ± 1	94 ± 1
D1 (24)	4 ± 7	21 ± 18	51 ± 13	64 ± 22	93 ± 2	91 ± 2
D10 (4)	7 ± 8	87 ± 5	95 ± 1	96 ± 1	96 ± 1	97 ± 1
D10 (24)	4 ± 8	78 ± 6	88 ± 3	82 ± 2	93 ± 2	93 ± 2
D10 (48)	4 ± 6	62 ± 10	74 ± 3	74 ± 3	85 ± 3	82 ± 4
D10 (168)	1 ± 1	7 ± 8	13 ± 13	12 ± 10	6 ± 9	26 ± 7

*Placebo from all MAD cohorts at respective nominal time points.

Safety

PRN2246 was well tolerated at all levels in the SAD portion of the study with no serious TEAEs. In the MAD portion of the study, all treatment related TEAE's for all combined dose groups were mild in nature with only diarrhea and headache occurring in >10% of all subjects. There were no treatment related clinically significant or drug-related changes in vital signs, ECG parameters, or laboratory values, except for one mild treatment related event of decreased platelet count in Part B for a participant that presented with low (NCS) platelet values at baseline trending downwards from Day 7. Study drug was withdrawn following dosing on Day 9 and platelet values thereafter were variable but tended to increase (from Day 10 to Day 18). The AE was considered resolved 15 days after onset. The frequency of PRN2246-related events of diarrhea was higher in the highest level dose group. There were no other clinically significant patterns of note in treatment related TEAEs, regardless of assigned treatment and single or repeat dose administration, and all treatment related TEAEs were mild.

Table C. PRN2246 Related TEAEs (MAD)

	7.5mg	15mg	30mg	60mg	90mg	ALL 2246	Placebo
Number of Subjects	8	8	8	8±	8	40	10
Treatment related TEAE [N]	4	2	3	2	5	16	2
- Diarrhea	1	1	2	0	5	9	1
- Headache	2	1	1	0	0	4	0

Conclusions

- Well tolerated at doses up to 120mg (SAD) and 90mg QD (MAD)
- Consistent PK with fast Tmax and rapid clearance
- High levels of peripheral BTK occupancy achieved at doses as low as 7.5mg
- PRN2246 exhibits BBB permeability and significant CNS exposure can be achieved
- Future studies evaluating PRN2246 in MS patients are planned

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4. This poster was reviewed by the Sanofi-Genzyme publications team.

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