The role of B cells in the pathophysiology of multiple sclerosis (MS) has been validated by the clinical results of B lymphocyte depletion with rituximab, an anti-CD20 antibody.1-3 BTK, the protein 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 inhibited BTK dependent inflammatory immune mechanisms in multiple immune cell types. Of relevance to MS, PRN2246 potently inhibits BTK-mediated B cell activation and maturation, and can inhibit BTK in microglial cells isolated from the central nervous system.

Figure A2. 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 assay. In a screen across 250 kinases, 12 of 250 kinases exhibited >90% inhibition at 1µM (Figure B).

Figure B. PRN2246 activity and selectivity.

**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 exposures. Modest accumulation was observed with multiple dosing.

Table A. Multiple Dose PRN2246 Pharmacokinetics (Day 10)

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>C₁₀₀ (ng/mL)</th>
<th>C₅₀ (ng/mL)</th>
<th>C₂₀ (ng/mL)</th>
<th>T₁/₂ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 mg QD</td>
<td>7.5 (7.0-10.0)</td>
<td>5.0 (4.0-7.0)</td>
<td>3.0 (2.0-5.0)</td>
<td>12.0 (11.0-13.0)</td>
</tr>
<tr>
<td>30 mg QD</td>
<td>15.0 (14.0-16.0)</td>
<td>10.0 (9.0-12.0)</td>
<td>6.0 (5.0-8.0)</td>
<td>12.0 (11.0-13.0)</td>
</tr>
<tr>
<td>60 mg QD</td>
<td>30.0 (29.0-33.0)</td>
<td>20.0 (19.0-22.0)</td>
<td>12.0 (11.0-13.0)</td>
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</tr>
</tbody>
</table>

**Assessment of BTK Occupancy**

• BTK Occupancy at Day 1 increases in a dose dependent manner with full occupancy at doses >50mg
• 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 C. PRN2246 exposure.**

**CSF Exposure**

CSF exposure was assessed via lumbar puncture at 2h. Peripheral BTK occupancy was assessed at various timepoints by an ELISA based readout using an irreversible probe.

**Figure D. CSF exposure of PRN2246 (Part C)**

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