**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 cytosine 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 synovium (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 252 other kinases. Cytokine 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 binding was completely reversible after denaturation of the target. Anti-CD4-induced human B cell proliferation (10%) and B cell CD69 expression (100%) were inhibited by PRN1008 with IC50 of 5 ± 2 nM and 0.2 ± 18 nM, respectively. PRN1008 did not block EGFR signaling in epithelial cells or TCM and calcium flux stimulated T cell activation. PRN1008 also did not block L-64 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-CO2 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.

**Conclusions**

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

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**Preclinical Characterization of PRN1008, a Novel Reversible Covalent Inhibitor of BTK that Shows Efficacy in a Rat Model of Collagen-Induced Arthritis**

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