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Target Engagement, Pathway Inhibition, and Efficacy Of The Bruton's Tyrosine Kinase (Btk) Inhibitor CC-292

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Abstract

Introduction

The B-cell receptor (BCR) and its downstream effectors have emerged as important therapeutic targets in B-cell malignancies. CC-292 is a novel, potent, covalent, and highly selective inhibitor of Btk ($IC_{50apparent}$ of 0.5 nM, k_{inact}/K_I ratio of 7.69×10^4 $M^{-1}s^{-1}$), that does not appreciably inhibit other kinases involved in BCR signaling (eg, IC_{50} Lyn kinase, 4401 nM) (Evans et al., *J Pharmacol Exp Ther*. 2013). Here, we report preclinical characterization and clinical data in CLL from a single-agent phase 1 dose-escalation trial of CC-292 in B-cell malignancies, with a focus on how target engagement and downstream events correlate with clinical activity.

Results

Pharmacodynamic effects of Btk inhibition by CC-292 can be monitored by occupancy of the Btk catalytic site, Btk autophosphorylation on Y223, and downstream phosphorylation of Plc-γ2 and Erk. We developed a sensitive (10 pg/mL lower limit of quantification) and quantitative assay to measure covalent binding of CC-292 to Btk (Evans et al., *J Pharmacol Exp Ther*. 2013), as well as Western and novel phos-flow assays to probe downstream signal transduction. These methods showed that CC-292 treatment blocks Btk autophosphorylation and downstream pathway activation in both tumor cells and human peripheral blood mononuclear cells (PBMCs). The extent of CC-292 binding to Btk correlated with its in vitro and in vivo effects. The occupancy assay demonstrated that CC-292 effectively targets Btk in tumor cell lines, PBMCs, spleen, and lymph nodes (LNs) in animal models, and in PBMC and lymph node samples from clinical trial subjects. In rats and non-human primates treated with CC-292, Btk occupancy in spleen and LNs was dose-dependent. Measured occupancy in rat spleen and axillary, mesenteric, and superficial cervical LNs was 94%, 92%, 90%, and 76% respectively, 4 hours (hrs) after a single 30-mg/kg dose. Interim data from the phase 1 CLL trial showed that PBMC Btk was completely occupied in the majority of subjects 4 hrs post-dose with both QD and BID dosing. Twenty-four hrs post-dose at 750 and 1000 mg QD, CC-292 exhibited 83% ± 17% Btk occupancy, whereas with BID dosing at 375 and 500 mg, occupancy was 94% ± 16% at the corresponding time point (12 hrs after the second dose). Thus, while both schedules achieved extensive and sustained Btk occupancy, residual free Btk levels were lower with the BID schedule, offering a rationale for an early trend towards more rapid nodal responses, lymphocytosis, and partial responses on the BID schedule observed to date in the phase 1 study. In the 10 clinical LN biopsies tested to date, no measurable levels of unoccupied Btk have been detected, although Btk protein was present as determined by Western blotting, showing that CC-292 was able to penetrate LNs and inhibit Btk in human subjects as it did in preclinical models. For monitoring downstream signal transduction, we developed reagents and assays including a phos-flow assay based on a novel rabbit monoclonal antibody to detect Btk pY223 levels in PBMC subsets. CC-292 effectively inhibited constitutive and induced phosphorylation of Btk and Plc-γ2 at low nanomolar concentrations. CC-292 also inhibited BCR activation and nurse-like cell–supported survival of CLL cells. Furthermore, CC-292 reduced CLL cell migration and actin polymerization in response to chemokines (CXCL12, CXCL13) and inhibited secretion of the chemokines CCL3 and CCL4 by CLL cells. These chemokines are essential for migration and retention of normal and neoplastic B cells in the marrow and secondary lymphatic tissues. Consistent with this preclinical data, CC-292 treatment resulted in rapid reductions in circulating CCL3 and CCL4 levels. In subjects treated at the 750 mg QD, 1000 mg QD, 375 mg BID, and 500 mg BID dose levels, plasma CCL3 was reduced from 99 ± 16 pg/ml before treatment to 28 ± 5 pg/ml (N = 48, mean ± SEM) at 24 hrs after the first dose, while CCL4 was reduced from 235 ± 59 pg/ml to 74 ± 16 pg/ml (N = 51).

Conclusions

These data demonstrate that CC-292 achieves significant and durable occupancy of Btk in vitro and in vivo, inhibits Btk-mediated downstream signaling events and chemokine production, and that these preclinical activities have translated into the clinic. Taken together, these results argue that Btk inhibition is necessary and sufficient for clinical activity in CLL. These emerging data support continued development of CC-292 for the treatment of B-cell malignancies.

Disclosures:

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