Introduction

- Bruton’s tyrosine kinase (BTK) is an essential component of normal and malignant B-cell function.
- Covalent BTK inhibitors have transformed the treatment of B-cell malignancies but are limited by off-target toxicity and acquired resistance, leading to eventual treatment disqualification and disease progression. Emerging evidence suggests that acquired resistance is mediated predominantly by BTK C481 substitution mutations at the covalent BTK inhibitors’ binding site. There is significant unmet clinical need for new treatment approaches that overcome acquired resistance and minimize toxicity.

LDXO-305 is a highly selective, non-covalent, next generation BTK inhibitor. Previously, we showed that LOXO-305 potently inhibited both wild-type (WT) BTK and BTK C481S-mediated kinase activity in enzyme and cell-based assays with nanomolar potency, and caused regression of BTK-dependent lymphoma tumors in mouse xenograft models. LOXO-305 was also more than 300-fold selective for BTK versus 370 other kinases and showed no significant inhibition of non-kinesin off-targets at 1 µM. In addition, preclinical ADME and pharmacokinetic experiments in two species demonstrated that LDXO-305 would likely have reasonable human exposure and sustained BTK C481S target coverage in patients at clinically achievable doses.

Kinetic analysis by SPR: WT biontarylated BTK 2.695 (BTN-BTK) and BTN-BTK C481S were purchased from Cama Biosciences. BTN-BTK C481R, and BTN-BTK C481T were expressed as N-terminal DYKDDDDK tagged, biontarylated proteins (~80 kDa) using a baculovirus expression system. The total signal was normalized by hypotonic lysis and the proteins were purified using DYKDDDDK tag antibody agarose. The equilibrium-binding affinities for targeted BTK inhibitors to BTK enzyme variants were determined by SPR using the Biacore T200. Purified BTN-BTK proteins were immobilized on a SensorAvid sensor chip. Five increasing concentrations of each inhibitor plus blank controls were analyzed using a single cycle kinetics program with no regeneration step. Individual injection association and dissociation times were 120 s each with a final dissociation event of 1800 s. Association dissociation rate constants were calculated by global fitting of the data to a 1:1 binding interaction model using T200 evaluation software v1.3.

Materials and Methods

- Potency study in cells: HEK293T cell lines transiently expressing WT BTK and BTK C481S substitution mutations were incubated with LDXO-305, bruton, or acalabrutinib for 30 min followed by the addition of orthovanadate. After 2 h, cells were lysed, and phosphorylated Y223 BTK and total BTK levels were detected by immunoblot (BTN WT, C481S, and C481T) or Mesoscale (C481R, C481F). Bands and MS signals were quantified, and the IC50 values calculated with GraphPad Primal.

Objectives

- Here we describe the cellular activity of LDXO-305 against BTK C481 substitution mutations, including mutations identified in patients with acquired resistance to covalent BTK inhibitors. We further determine equilibrium-binding affinities for LDXO-305 for diverse mutant BTK enzymes and compare these activities to other clinically available BTK inhibitors.

Results

- Surface Plasmon Resonance Binding Assay

- Western blot analysis: HEK293T cell lysates after transfection with WT and mutant forms of BTK, C481S, and C481T were incubated with various concentrations of LDXO-305. The membrane was reduced by 50%, while C481S showed weakly catalytic activity. The C481S phosphorylation was detected for C481R and not C481T. Western blot analysis shows that the phospho signal was normalized to total BTK. A bar graph showing the relative amount Y223 phosphorylation inBTN WT and mutant forms. Western blot analysis shows that the phospho signal was normalized to total BTK. Western blot analysis shows that the phospho signal was normalized to total BTK.

Conclusion

- The next generation, non-covalent, highly selective BTK inhibitor LDXO-305 potently inhibited the cellular activity of BTN-BTK C481S, T, and R mutations and displayed strong equilibrium binding to WT BTK and transformed BTK C481 substitution mutations. Together with high selectivity and significant BTK target coverage in vivo, these results indicate that LDXO-305 may overcome acquired resistance to covalent BTK inhibitors in patients without significant off-target toxicity. A phase 1 clinical trial for LDXO-305 is currently underway.

References