Introduction

- Bruton’s tyrosine kinase (BTK) is an essential component of normal and malignant B-cell receptors.
- 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 discontinuation 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.1

- LOXO-305 is a highly selective, non-covalent, next generation BTK inhibitor. We previously showed that LOXO-305 potently inhibited both wild-type (WT) BTK and BTK C481S-mediated kinase activity in vitro and in 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 enzymes and compare these activities to other clinically available BTK inhibitors.2

Objectives

- LOXO-305 inhibits BTK C481 substitution mutations
- Kinetic analysis by SPR: WT biont BTK 2.69 (BTN-BTK) and BTK-BTK C481S were purchased from Cama Biosciences. BTK-BTK C481R, and BTK-BTK C481T were expressed as N-terminal DYKDDDK tagged, biont proteins (80 kDa) using a baculovirus expression system. The recombinant protein was purified by hypotonic lysis and the proteins were purified using DYKDDDK tag antibody agarose. The equilibrium-kinetic 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 Series S Streptavidin 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 v3.1.5

Results

- Evaluation of the potency of LOXO-305, ibrutinib and acalabrutinib on inhibiting BTK WT autophosphorylation. (A) Representative Western blot membranes for total and phospho-BTK. (B-C) WT BTK autophosphorylation was normalized to total BTK. (B) Dose-response curves expressed as percentage of the DMSO control. (C) Table with average IC50 values and SD.

Materials and Methods

- Potency study in cells: HEK293T cell lines transiently expressing WT BTK and BTK C481 substitution mutations were incubated with LOXO-305, brutinib 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 (BTK WT, C481S, and C481T) or Moses/Actin (C481B). Bands and MS signals were quantified, and the IC50 values calculated with GraphPad Prism.

Conclusions

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

References


Disclosures

All authors are employees of Lexicon Oncology, Inc., a wholly owned subsidiary of Eli Lilly and Company.