LOXO-305, a next generation non-covalent BTK inhibitor, for overcoming acquired resistance to covalent BTK inhibitors

Barbara Brandhuber,1 Eliana Gomeza,1 Steven Smith,2 Todd Earv,3 Stacey Spencer,1 S. Michael Rothenberg,1 Steven Andrews1

1Loxo Oncology, Boulder, CO, United States 2Loxo Oncology, South San Francisco, CA, United States

Abstract

LOXO-305 is a next generation, non-covalent BTK inhibitor that can overcome acquired resistance to covalent BTK inhibitors in preclinical models, with minimal off-target kinase and non-kinase inhibitory activity.

Materials and methods

Kinase profiling: radiolabeled ATP kinase activity assays were run at the K_i, ATP concentration for each enzyme. LOXO-305 at 1 µM was tested against 371 wild-type kinases. Biochemical inhibition in duplicate (or greater replicate number) 10-point dose response curves were used to determine the biochemical IC_{50} values. 1Lyte kinase assays (ThermoFisher) were used to determine EGFR and Jan 1 IC_{50} values. HEK293 cell lines stably expressing BTK and BTK C481S were generated using standard lentiviral transfection/methods. Ramos RA1 cell line was obtained from ATCC. TMD8 cell line was licensed from Tokyo Medical and Dental University. In the chemoproteomics study, LOXO-305 was profiled in human peripheral mononuclear cells competitively inhibited the binding of the chemoproteomic nucleotide acyl phosphates of ATP and ADP, which covalently react with protein kinases on tyrosine residues in the ATP site. Binding of LOXO-305 to 180 kinases in PBMCs was determined by comparing the ATP and ADP probe labeled peptide signals from LOXO-305 treated PBMCs to untreated PBMCs. Human liver microsome stability was evaluated in a pool from 150 donors. Human hepatocyte stability was evaluated in mixed gender cryopreserved hepatocytes from 150 donors. In the chemoproteomics study, LOXO-305 was shown with identifying chemoproteomics probe peptide and IC_{50} values were calculated using a 4-parameter fit in GraphPad Prism 7.04 software.

Results

LOXO-305 is predicted to have low human clearance and high permeability in human in vitro assays is predicted to achieve high BTK and BTK C481S target coverage in humans at reasonable doses based on these promising results, the first-in-human clinical trial of LOXO-305 in patients who have progressed on prior covalent BTK inhibitors is planned.