LOXO-101, a pan TRK inhibitor, For The Treatment Of TRK-driven Cancers

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Abstract #391

Abstract

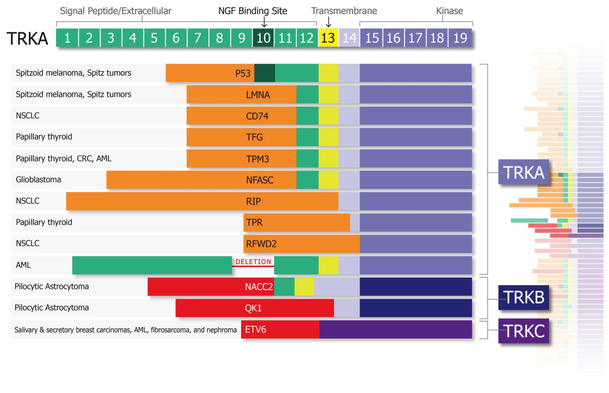
Background: TRK family of neurotrophin receptors, TRKA, TRKB, and TRKC (encoded by *NTRK1, NTRK2,* and *NTRK3* genes, respectively) and their neurotrophin ligands regulate growth, differentiation and survival of neurons. Translocations involving the *NTRK* kinase domain, mutations involving the TRK ligand-binding site, amplifications of *NTRK,* TRK splice variants, and autocrine/paracrine signaling are described in a diverse number of tumor types and may contribute to tumorigenesis. Recently *NTRK1* fusions were described in a subset of adenocarcinoma lung cancer patients (Vaishnavi, 2013). LOXO-101 is a potent, ATP-competitive TRK inhibitor with IC_{50}s in low nanomolar range for inhibition of all TRK family members in binding and cellular assays, with 100x selectivity over other kinases.

Methods: In vitro and in vivo evaluations including PK/PD correlations, pharmacokinetic (PK) and drug metabolism characterization, and non-clinical safety evaluation were conducted with standard procedures.

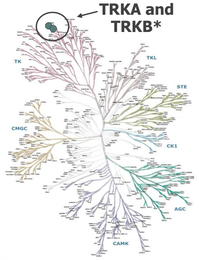
Results: In rat and monkey studies LOXO-101 demonstrated 33-100% oral bioavailability and 60-65% plasma protein binding. It had low brain penetration, and was well tolerated in 28 day (d) GLP toxicology studies and was neither an inducer nor inhibitor of human CYP3A4. In a constitutively-active TRKA murine tumor model developed to investigate activity of TRK-inhibitory drugs, a single dose (30 mg/kg) of LOXO-101 reduced tyrosine phosphorylation of TRKA and downstream signal transduction (pERK) in the tumor >80%. LOXO-101 was well tolerated up to 200 mg/kg/day for 14 d in this model. Delays of tumor growth were observed with the lower dose LOXO-101, with sustained inhibition of tumor growth (TGI) for up to 6 d after discontinuation of treatment with the higher doses. Crizotinib at 50 mg/kg was limited to 50% inhibition of pTRKA in tumor-bearing mice and was ineffective at blocking tumor growth.

Conclusions: LOXO-101 is an orally bioavailable first-in-class kinase inhibitor developed specifically for TRK inhibition with favorable PK and drug metabolism properties in animals. LOXO-101 has demonstrated potent inhibition of TRKA in tumor bearing animals and was effective in inhibiting tumor growth in a TRKA driven malignancy. The ability to achieve concentrations above IC_{80} with this selective inhibitor may provide an improvement over multikinase therapy for TRK-driven malignancies. A Phase 1 clinical study has been initiated to determine its PK, PD, safety and anti-tumor activity in humans.

TRK Oncogenes



LOXO-101 Specificity



**TRKC not tested in this panel*

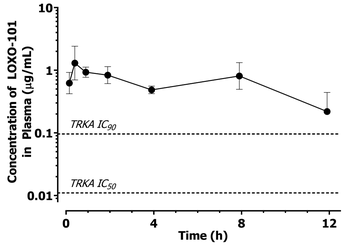
LOXO-101 is highly selective for TRKA, TRKB, and TRKC

- No inhibition of >200 other kinases at 1000 nM
- Weak inhibition of TNK2 (IC_{50} > 1000 nM)
- >100-fold selective for other kinases
- 1,000-fold selective for >75 non-kinase targets

LOXO-101 Selected Properties

PROPERTY	DESCRIPTION
Potency	2 to 20 nM potency against TRKA, TRKB, and TRKC (cell & enzymatic)
Selectivity	>100 fold over other kinases >1000 fold over >75 diverse mammalian targets
ADME	Oral bioavailability 30-100% in animals, metabolized by CYP3A4, low to moderate clearance across species
Protein Binding	Low to Moderate - 60% (mouse), 63% (rat), 61% (dog), 67% (monkey), and 69% (human)
Safety	No relevant hERG inhibition No preclinical QT findings (rat, dog, and monkey)
Dosing	Phase 1: Oral, continuous 28-day cycles
Toxicity	Wide safety margin at expected therapeutic doses

Pharmacokinetics of LOXO-101 in Mice

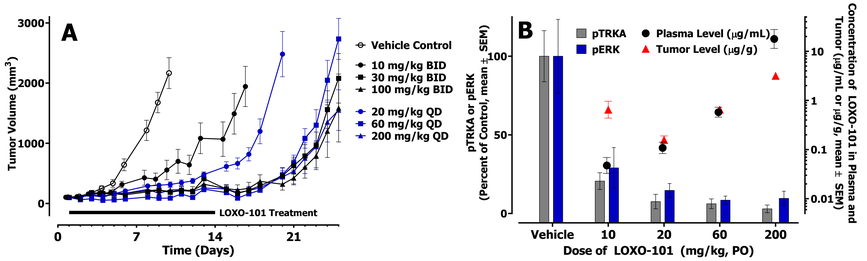


Mice were exposed to LOXO-101 for ≥12 hours after a 30-mg/kg oral dose

Unbound LOXO-101 concentrations in plasma were well above its IC_{50} and IC_{90} for inhibition of TRKA

LOXO-101 was formulated in Labrafac® and administered orally (30 mg/kg, single dose) to male C3H/HeJ mice. LOXO-101 concentrations in plasma were determined by LC-MS/MS (AB Sciex 4000 Q Trap). IC_{50} and IC_{90} were determined by a fluorescence resonance energy transfer (FRET) binding assay. LOXO-101 potency in whole-cell assays was similar to its potency in the binding assay.

Dose- and Schedule-Dependent Inhibition of Tumor Growth and pTRKA in 3T3-TRKA Allografts

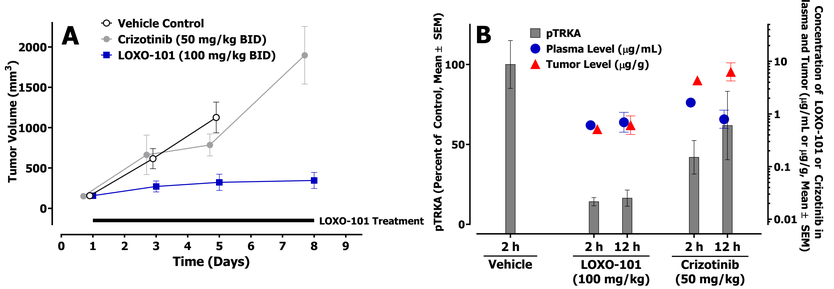


(**A**) LOXO-101 caused inhibition of tumor growth at doses of 20 to 200 mg/kg/day

(**B**) Inhibition of tumor growth was associated with dose-dependent reduction in pTRKA and pERK

The delta-Ig2-TRKA NIH-3T3 tumor cell line was constructed by transfecting murine 3T3 fibroblasts with altered gene. Deletion of the Ig2 domain (amino acids 303-377) confers constitutive activation (Reuther et al., 2000). 3T3-TRKA tumor allografts were staged at ~100 mm^3 in nu/nu NCr mice, which were treated with LOXO-101 PO at 20-200 mg/kg total daily dose. A) Tumor Growth Inhibition – LOXO-101 was administered for 14 days and tumor volume measured by digital caliper; B) PK/PD – Following a single oral dose, tumors were harvested at 2 hours after dosing and tested for pTRKA by ELISA (R&D Systems) or pERK (Cell Signaling Technology) by Western blot and drug concentrations in tumor and plasma were determined by LC-MS/MS.

LOXO-101 Demonstrates Greater Tumor Growth Inhibition and pTRKA Reduction than Crizotinib



(**A**) TGI with LOXO-101, whereas crizotinib was ineffective

(**B**) TGI by LOXO-101 was associated with a reduction in pTRKA

3T3-TRKA tumor allografts were staged at ~100mm^3 in nu/nu NCr mice, which were treated with LOXO-101 (100 mg/kg BID, PO) or crizotinib (50 mg/kg BID, PO). A) Tumor Growth Inhibition – Both drugs were administered for 8 days and tumor volume measured with a digital caliper; B) PK/PD – Following a single oral dose, tumors were harvested and tested for pTRKA by ELISA (R&D Systems) and drug concentrations determined by LC-MS/MS.

Summary

Rearrangements in *NTRK1*, *NTRK2*, and *NTRK3* that lead to constitutively-active TRKA, TRKB, and TRKC receptors are oncogenic and prevalent in a wide array of tumor types, including lung adenocarcinoma, thyroid, head and neck cancer, glioblastoma, and others

LOXO-101 demonstrates potent and highly-selective inhibition of TRKA, TRKB, and TRKC over other kinase- and non-kinase targets

LOXO-101 demonstrated robust inhibition of tumor growth in xenograft models, acceptable pharmaceutical properties, and safety in nonclinical models

These data support the use of LOXO-101 to investigate the therapeutic potential of a highly-selective TRKA/B/C inhibitor