**Abstract**

Background: TRK family of neurotrophin receptors, TRKA, TRKB, and TRKC (encoded by *NTRK1, NTRK2* and *NTRK3*, respectively) and their neurotrophins (NGF, BDNF and NT-3) are involved in cell proliferation, differentiation and survival of neurons. Transformations involving the NTRK gene family are associated with several human cancers. LOXO-101 is a highly selective, orally available small-molecule kinase inhibitor, with a low nanomolar range for inhibition of TRK family members in binding and cellular assays, with >1000 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 10-day and 14-day studies LOXO-101 demonstrated ≥2100% bioavailability and 60-65% plasma protein binding. It had low brain penetration, and was well tolerated in 28 day (QD) toxicology studies in rats and dogs. LOXO-101 is a constitutively active TRK inhibitor with IC₅₀ in the low nanomolar range for inhibition of all TRK family members in binding and cellular assays, with 100x selectivity over other kinases.

Conclusions: LOXO-101 is a highly selective small-molecule inhibitor developed specifically for TRK inhibition with favorable PK and drug metabolism properties in animals. LOXO-101 has demonstrated potent inhibition of TRK in tumor bearing animals and was effective in inhibiting tumor growth in a TRK driven malignancy. The ability to achieve concentrations above IC₅₀ 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, safety and anti-tumor activity in humans.

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

The nude rat 3T3-TRKA™ (TRKA) xenograft model was established by subcutaneously implanting 3T3 fibroblasts with altered gene expression of the TRKA-driven gene BDNF (Räikkönen et al., 2001). 3T3 TRKA tumor allorafts were staged of LOXO-101 (100 mg/kg, PO) using a single oral dose or 3 times daily (TID) for 14 days. 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). Following a 3 times daily dosing regimen, tumor and plasma were harvested at 12 hours (H) after dosing. For both groups, the tumor level and plasma concentration of LOXO-101 were determined using LC-MS/MS. IC₅₀ values determined by a dose effect binding assay. IC₅₀ in whole-cell assays was similar to its potency in the binding assay.

**Pharmacokinetics of LOXO-101 in Mice**

Mice were exposed to LOXO-101 for 11.2 hours after a 30-mg/kg oral dose. Untouched LOXO-101 concentrations in plasma were well above its IC₅₀, and IC₆₀ for inhibition of TRKA

**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 cancers, glioblastoma, and others.

LOXO-101 demonstrated 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 pharmacological 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.