The development of LOXO-195, a second generation TRK kinase inhibitor that overcomes acquired resistance to first generation inhibitors in patients with TRK-fusion cancers

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Introduction

TRK in cancer

Oncogenic NTRK gene fusions occur at low frequency, but in a wide spectrum of tumor types in patients. Early clinical trials of LOXO-101, a specific TRK tyrosine kinase inhibitor (TKI), and entrectinib, a multi-kinase inhibitor (MKI) with anti-TRK activity, have demonstrated tumor responses in TRK-fusion patients. Unfortunately, acquired resistance has been reported in entrectinib-treated patients, mediated by secondary mutations in the TRK fusion that may directly interfere with drug binding. Although resistance mutations have not yet been reported in LOXO 101-treated patients, entrectinib-resistant mutations may be cross-resistant to LOXO-101.

Two classes of acquired resistance mutations have been reported in patients to date: substitutions in the kinase "solvent front" (TRKA G595R, TRKC G623R) or the "xDFG" motif (TRKA G667C). No clinically available TRK inhibitors have been shown to overcome resistance mutations that have been reported in patients. Notably, homologous mutations in other fusion kinases (e.g. ALK, ROS1) confer acquired resistance to the relevant first generation TKIs (e.g. crizotinib); solvent-front mutations in particular are poorly addressed by currently available second generation inhibitors (e.g. ceritinib, alectinib).

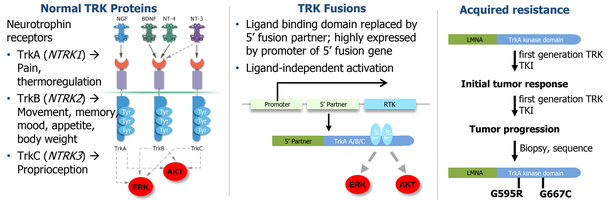


Figure 1. *Left* Normal TRK biology. *Middle* Chromosomal rearrangements produce oncogenic TRK fusions that activate the TRK kinase. *Right* Acquired resistance to first generation TRK TKIs.

We set out to identify a second generation TRK inhibitor that is unaffected by acquired resistance mutations identified in patients, while maintaining LOXO-101-like selectivity and excellent drug-like properties. Here, we present LOXO-195, a second generation, potent and specific TRK TKI with unique activity against acquired resistance mutations, including difficult-to-treat substitutions in the solvent front.

Methods

- LOXO-195 was identified as a potent and selective second generation TRK inhibitor with activity against wild-type TRK kinases and key resistance mutations, with high selectivity and oral bioavailability, and favorable pharmacokinetics (PK) in animals.
- *In vitro* and *in vivo* evaluations, including enzyme and cell-based assays, PK/PD (pharmacodynamics) correlations, drug metabolism characterization, and non-clinical safety evaluation were performed using standard methods.
- Efficacy and PK/PD studies were performed with TRK-dependent tumor models, including allografts of NIH 3T3 cells expressing a delta-Ig2-TRKA (ΔTRKA) activating mutation, without/with the G595R and G667C resistance mutations, in accordance with IACUC and Guide for Laboratory Animal Care and Use guidelines.

Results

X-ray crystal structures and models of TRK proteins and acquired resistance mutations

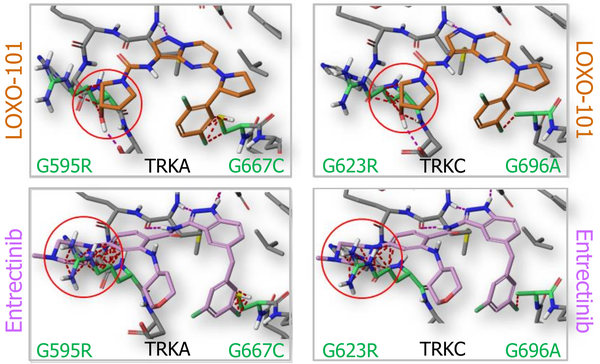


Figure 2. Structural modeling of entrectinib and LOXO-101 interactions with TRKA-G595R and TRKC-G623R (solvent front), and TRKA-G667C and TRKC-G696A (xDFG). Red circles indicate clashes.

LOXO-195 possesses high potency against TRK resistance mutations

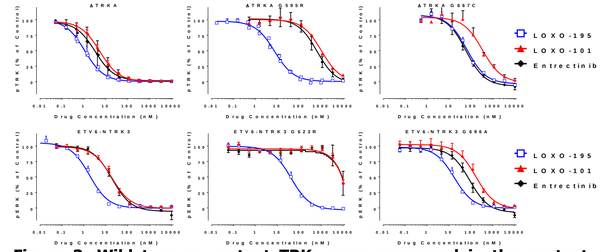
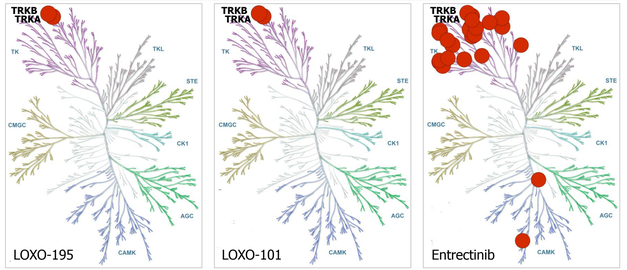


Figure 3. Wild type or mutant TRKs were expressed in the context of ΔTRKA or ETV6-NTRK3. For ΔTRKA constructs, inhibition of signaling was determined by examining pTRK levels in cell lysates by ELISA assay after treatment of cells with the indicated agents for one hour (*upper*). Since reagents are not available to evaluate pTRK in the context of the ETV6-NTRK3 fusion, pERK levels by flow cytometry were used as a downstream marker of TRK activation. (*lower*). n≥3.

LOXO-195 displays high selectivity across the kinome



Kinase	LOXO-195 % control
TRKA	0.5
TRKB	0
EGFR	100
KIT	100
Met	100
PDGFRalpha	100
PDGFRbeta	100
KDR (VEGFR2)	100

Figure 4. *Upper* Greater than 100-fold selectivity was observed for > 95% of 228 purified kinase domains in radiometric kinase assays for both LOXO-101 and LOXO-195 when tested at 1 μM. Entrectinib is shown for comparison. *Lower* Percent of control values for select kinases commonly inhibited by MKIs. Note: TRKC was not included in this analysis.

LOXO-195 is selectively cytotoxic to TRK-fusion cells

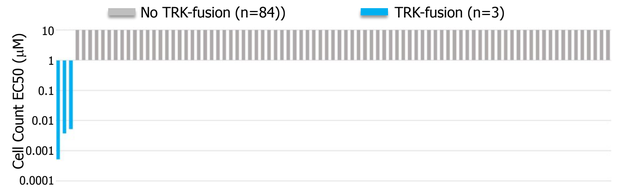


Figure 5. Each cell line was treated with 10 concentrations of LOXO-195 in triplicate for 72 hours, followed by DAPI staining and cell counting.

Dose-dependent inhibition of TRK and tumor growth in diverse activated TRK mouse models

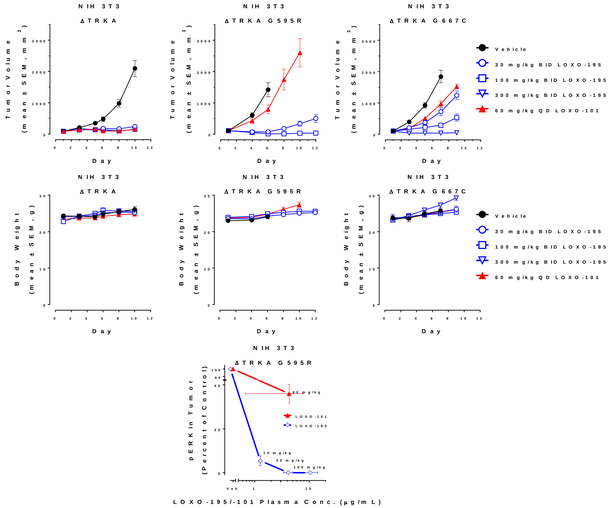


Figure 6. Twice daily treatment with LOXO-195, but not LOXO-101, caused dose-dependent inhibition of tumor growth for all ΔTRKA allografts, including ΔTRKA (wild type), solvent-front (ΔTRKA-G595R) and xDFG (ΔTRKA-G667C) *(upper)*, without decreasing body weight (*middle*). pERK levels in tumor lysates after dosing NIH 3T3-ΔTRKA-G595R allografts with LOXO-101 or LOXO-195 were determined by immunoblot and compared to drug plasma levels (*lower).*

Summary

We have identified LOXO-195 as a second generation TRK inhibitor with high oral bioavailability and favorable PK in animals. LOXO-195 demonstrated potent inhibition of TRK fusions, including critical acquired resistance mutations, in enzyme and cellular assays, with minimal activity against other kinases. In diverse TRK fusion mouse models, LOXO-195 inhibited phospho-ERK and caused dramatic tumor growth inhibition, superior to first generation TRK inhibitors, without significant toxicity. Remarkably, LOXO-195 could overcome challenging solvent-front resistance mutations identified in both TRKA and TRKC fusions that have arisen in patients treated with entrectinib. Therefore, LOXO-195 has the potential to address a critical unmet clinical need for patients whose tumors have progressed after treatment with first generation TRK inhibitors. LOXO-195 is anticipated to enter the clinic in 2017.