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


Introduction

TRK in cancer

Oncoytic 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 response 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.

LOXO-195 possesses high potency against TRK resistance mutations

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 kinase and key resistance mutations, with high selectivity and oral bioavailability, and favorable pharmacokinetics (PK) in animals.
• In vitro and in vivo evaluations, including resistance and acquired-resistant (pharmacodynamics) correlations, drug metabolism characterization, and non-clinical safety evaluation were performed using standard protocols.
• Efficacy and PK/PD studies were performed with TRK-dependent tumor models, including allografts of NIH 3T3 cells expressing a deltaNp75TRKA (Δp75TRKA) activating mutation, without 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 3. Wild type or mutant TRKs were expressed in the context of Δp75TRKA or ΔETV6-TRK oncogenic constructs. Expression levels were 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-ΔTRK2 fusion, pTRK levels by flow cytometry were used as a downstream marker of TRK activation. (Δover), n=3.

LOXO-195 displays high selectivity across the kinase

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

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 Δp75TRKA (wild type), -Δp75TRK (Δp75TRKA), and NTRK1 (ΔG667C (Δp75TRKA)) fusions, including acquisition of a 100 molar dose of NTH-37-Δp75TRKA-xenograft allografts with LOXO-195 or LOXO-101 were determined by immunoblot and compared to drug plasma levels.

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 kinase activity and acquired resistance mutations, in xenograft and cellular assays, with minimal activity against other kinases. In diverse TRK fusion mouse models, LOXO-195 inhibited phosphorylation and caused dramatic tumor growth inhibition, superior to first generation TRK inhibitors, without significant toxicity. Remarkably, LOXO-195 could overcome challenging Δp75TRKA-resistant 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 need for patients whose tumors have progressed after first generation TRK inhibitors. LOXO-195 is anticipated to enter the clinic in 2017.