The development of LOXO-292, a potent, KDR/VEGFR2-sparing RET kinase inhibitor for treating patients with RET-dependent cancers

B. Brandhuber1, J. Haas1, B. Tuch2, K. Ebata3, K. Boutiana4, E. McFadden5, L. Williams4, S. Winksw, E. Brown, M. Bunkhard6, N. Nandar, R. Hamori, F. Sullivan, L. Hanson5, T. Morales6, G. Vigors7, R.D. Wallace8, J. Blake8, S. Smith2, S. Andrew9, G.M. Rothbard10,11,12,13,14

1Array BioPharma, Drug Discovery, Boulder, CO, USA, 2Loxo Oncology, Research and Development, Stanford, CT, USA

Introduction

RET in cancer
Activating mutations and fusions of the RET receptor tyrosine kinase have been identified in several cancer types, including thyroid, lung, breast and colon cancer. Furthermore, tyrosine kinase inhibitors (TKIs) that inhibit RET have activity in patients with RET-dependent cancers. However, current TKIs are only moderately potent at inhibiting TRKs and KIT, and their selectivity for RET is limited (e.g. KDR/VEGFR2) and poorly inhibit anticipated secondary resistance (e.g. gatekeeper) mutations.

LOXO-292 Targets Diverse RET Activating Alterations and Anticipated Acquired Resistance Mutations

• LOXO-292 was selected as the lead candidate by determining: (1) activity against KIF5B- and CDDQ-RET fusions found in lung cancer, HR1ET and C364H substitutions seen in medullary thyroid cancer and V604M gatekeeper resistance mutations; (2) selectivity against a broad panel of kinase and other anti-tumor agents; (3) high oral bioavailability; and (4) favorable pharmacokinetics (PK) in animals.
• In vitro and in vivo evaluation of drug candidate, including enzyme and cell-based assays.
• Activity in vivo was validated by measuring PK target inhibition and efficacy in RET-dependent tumor models, including allografts of engineered for TERT, cancer cell line xenografts and patient-derived mouse xenografts (PDX), in accordance with IACUC guidelines and the Guide for Laboratory Animal Care and Use.

Methods

Results

LOXO-292 achieves high selectivity for KDR/VEGFR2

LOXO-292 has the potential to cause dose-dependent inhibition of phospho-RET and tumor growth in diverse RET mouse models

Figure 4. Left LOXO-292 at 0.1 µM demonstrated >100-fold selectivity for >95% of 228 purified kinase domains in radiometric kinase assays. Vandetanib is shown for comparison. Right Since enzyme assays of KDR/VEGFR2 (v939) and KIF5B-RET (v804m) may underestimate activity in patients, the IC50 values for phospho-RET and phospho-KDR in HEK293 cells expressing KIF5B-RET or KDR were determined for hundreds of compounds (circles) after treatment for one hour. While both MKIs are more potent against KDR than KIF5B-RET (KDR(IC50, >100), LOXO-292 is >100-fold more active against KIF5B-RET than KDR.

Figure 5. Each human cancer cell line (n=87) was treated with 10 concentrations of each inhibitor in triplicate, following by DAPI staining and cell counting.

Figure 6. Twice daily treatment with LOXO-292 (blue curves) caused dose-dependent inhibition of tumor growth for all RET-dependent tumor models, including N1163 KIF5B-RET (+/+ and +/−) allografts and CCDS6-RET (−/−) s.c. xenografts in immunocompromised NOD-SCID (−/−) mice. LTC and 2-za (lung cancer CCDS6-RET) and fatty (medullary thyroid cancer C634R-RET) xenografts (midline, panels 5 & 6): without decreasing body weight (midline, panel 7). By comparison, both cabozantinib (green) and ponatinib (red) were less effective against the V804M gatekeeper mutation (upper, panel 4) and caused tumor regression in xenografts with prolonged dosing (middle, panel 7). pRET levels in tumors lysates after dosing NID 3T3 KIF5B-RET (−/−) V804M allografts with LOXO-292 (or vehicle analog) were determined by immunoblot and compared to drug plasma levels (lower).

Summary

We have developed LOXO-292, a potent and specific RET kinase inhibitor with favorable pharmacological properties and potent activity against diverse RET-dependent cancers in vitro and in vivo, including founder genetic alterations and resistance mutations that may otherwise present treatment challenges. LOXO-292 is selective for RET with specific binding to KIF5B-RET and KDR/VEGFR2, with significant sparing of other kinase and non-kinase anti-targtets, it is predicted to robustly inhibit RET in patients at clinically relevant doses, and therefore offers the potential for more effective and safe treatment of patients with RET-dependent cancers. LOXO-292 is anticipated to enter the clinic in early 2017.

References
