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

ENA-0490/Poster No. 441

B. Brandhuber[1], J. Haas[1], B. Tuch[2], K. Ebata[2], K. Bouhana[1], E. McFaddin[1], L. Williams[1], S. Winski[1], E. Brown[1], M. Burkhard[1], N. Nanda[2], R. Hamor[1], F. Sullivan[1], L. Hanson[1], T. Morales[1], G. Vigers[1], R.D. Wallace[1], J. Blake[1], S. Smith[2], S. Andrews[1], S.M. Rothenberg[2]. [1]Array BioPharma, Drug Discovery, Boulder, CO, USA, [2]Loxo Oncology, Research and Development, Stamford, 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 carcinoma. Furthermore, tyrosine kinase inhibitors (TKIs) that inhibit RET have activity in patients with RET-dependent cancers. However, current TKIs are only moderately potent against RET, cause toxicity through stronger inhibition of other kinases (e.g. KDR/VEGFR2) and poorly inhibit anticipated secondary resistance (e.g. gatekeeper) mutations.

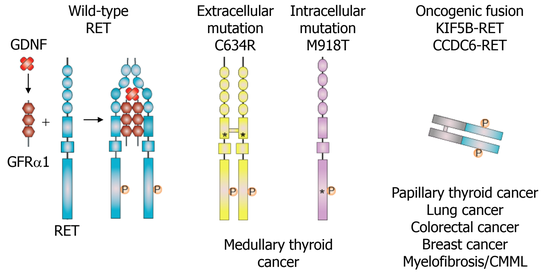


Figure 1. RET alterations in human cancers lead to ligand-independent RET dimerization (e.g. C634R), kinase activation (e.g. M918T) or fusion-induced dimerization (e.g. KIF5B-RET). Figure modified from Mulligan, Nat Rev Cancer (2014) 14;3: 173-185.

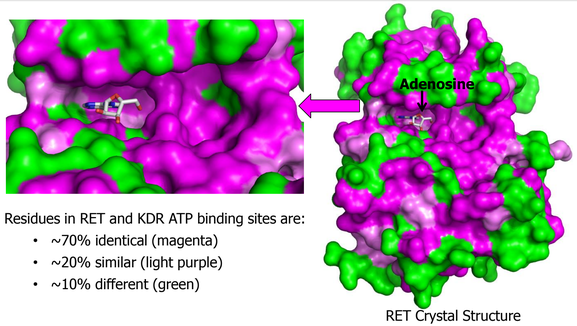
We set out to design an inhibitor that targets diverse RET alterations, with significant sparing of anti-targets and excellent drug-like properties. Here, we present LOXO-292, a potent and specific RET inhibitor with activity against diverse RET fusions and activating mutations and significant sparing of KDR/VEGFR2.

Methods

- LOXO-292 was selected as the lead candidate by determining: (1) activity against KIF5B- and CCDC6-RET fusions found in lung cancer, M918T and C634W substitutions seen in medullary thyroid cancer and V804L/M gatekeeper resistance mutations; (2) selectivity against a broad panel of kinase and other anti-targets; (3) high oral bioavailability, and (4) favorable pharmacokinetics (PK) in animals.
- In vitro and in vivo evaluations, including enzyme and cell-based assays and PK/PD (pharmacodynamics) correlations, drug metabolism characterization, and non-clinical safety evaluation were performed using standard methods.
- Activity in vivo was validated by measuring PD target inhibition and efficacy in RET-dependent tumor models, including allografts of engineered NIH 3T3 cells, cancer cell line xenografts and patient-derived mouse xenografts (PDX), in accordance with IACUC guidelines and the Guide for Laboratory Animal Care and Use.

Results

RET and KDR/VEGFR2 possess a high degree of structural similarity

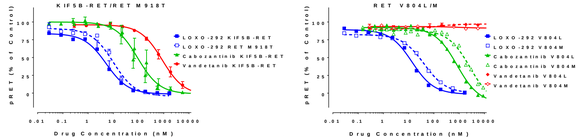


Residues in RET and KDR ATP binding sites are:

- ~70% identical (magenta)
- ~20% similar (light purple)
- ~10% different (green)

Figure 2. X-ray crystal structure of the RET kinase domain. Residues are colored by the degree of KDR/VEGFR2 identity/similarity/difference. With only ~10% of residues distinctly different between the two kinases in the ATP/drug binding site, avoiding KDR/VEGFR2 is challenging.

LOXO-292 targets diverse RET activating alterations and anticipated acquired resistance mutations



RET Mutation	Enzyme Fold vs. WT RET	Cellular Fold vs. WT RET
WT	1	1
V804M	2	8
V804L	2	3
A883F	4	ND
M918T	2	2
S891A	2	ND

Figure 3. *Upper* pRET levels in cells were determined by in-cell Western assay after treatment of the indicated HEK-293-RET cells with LOXO-292 (blue), cabozantinib (green) or vandetanib (red) for one hour, n=2-4. *Left* KIF5B-RET and RET M918T. *Right* RET V804L and RET V804M. *Lower* The table summarizes fold IC_{50} values, vs. wild-type (WT) RET kinase (enzyme) or KIF5B-RET (cellular), for LOXO-292 against RET mutations found in medullary thyroid cancers.

LOXO-292 achieves high selectivity for KDR/VEGFR2

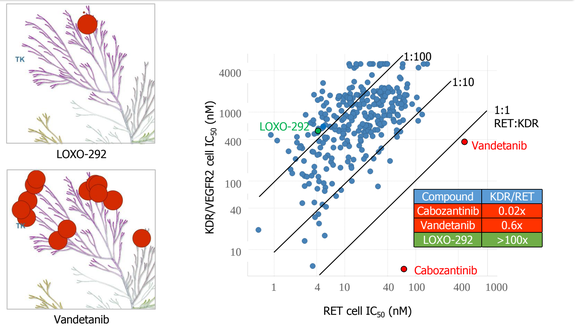


Figure 4. *Left* LOXO-292 at 0.1 μM demonstrated > 100-fold selectivity for > 95% of 228 purified kinase domains in radiometric kinase assays. Vandetanib is shown for comparison. *Right* Since enzyme assays of KDR/VEGFR2 may underestimate selectivity in patients, the IC_{50} values for phospho-RET and phospho-KDR inhibition 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/RET IC_{50} <1), LOXO-292 is >100-fold more active against KIF5B-RET than KDR.

LOXO-292 is selectively cytotoxic to RET-altered cells

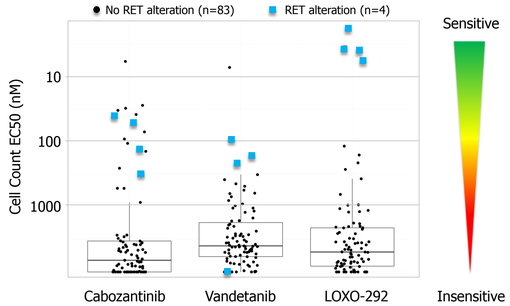


Figure 5. Each human cancer cell line (n=87) was treated with 10 concentrations of each inhibitor in triplicate, following by DAPI staining and cell counting.

Dose-dependent inhibition of phospho-RET and tumor growth in diverse RET mouse models

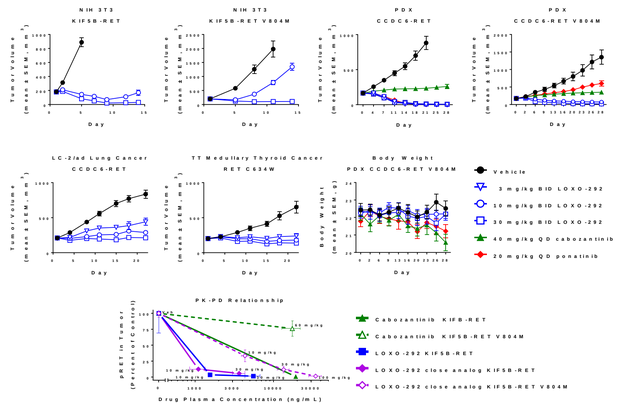


Figure 6. Twice daily treatment with LOXO-292 (blue curves) caused dose-dependent inhibition of tumor growth for all RET-dependent tumor models, including NIH 3T3 KIF5B-RET (-/+ V804M) allografts and CCDC6-RET (-/+ V804M) PDXs *(upper, panels 1-4);* and LC-2/ad (lung cancer CCDC6-RET) and TT (medullary thyroid cancer RET C634W) xenografts *(middle, panels 5-6),* without decreasing body weight (*middle, panel 7*). By comparison, both cabozantinib (green) and ponatinib (red) were less effective against the V804M gatekeeper mutation (*upper, panel 4*) and caused body weight loss with prolonged dosing (*middle, panel 7*). pRET levels in tumor lysates after dosing NIH 3T3 KIF5B-RET (-/+ V804M) allografts with LOXO-292 (or close 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 pharmaceutical properties and potent activity against diverse RET alterations in vitro and in vivo, including founder genetic alterations and resistance mutations that may arise following treatment with multikinase inhibitors. Together with significant sparing of other kinase and non-kinase anti-targets, 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.