Identification and characterization of highly potent and selective RET inhibitors for the treatment of RET-driven cancers

Introduction

Activating mutations and oncogenic fusions of the RET receptor tyrosine kinase have been identified in multiple tumor types, including thyroid, lung, breast, and colon carcinoma. Furthermore, tyrosine kinase inhibitors (TKIs) with anti-RET activity have produced clinical responses in patients whose tumors harbor RET alterations. However, currently available RET inhibitors were initially developed to target kinases other than RET and are only moderately potent against RET, inhibit multiple kinases other than RET or poorly inhibit secondary resistance mutations (e.g. gatekeeper mutations) common to other TKIs.

We have discovered novel, potent and selective RET inhibitors. The resulting compounds exhibit nanomolar potency against wild type RET and select RET mutants, including the KIF5B-RET fusion and CCDC6-RET gatekeeper mutants, in both enzyme and cellular assays, with minimal activity against highly related kinases. The activity of representative compounds and that of related analogs in relevant in vitro and in vivo models is presented.

Methods

• R001 and R002 were identified as potent and selective RET inhibitors with high oral bioavailability and favorable pharmacokinetic (PK) properties in animals.
• In vitro and in vivo evaluations, including enzyme and cell-based assays, PK/PD (pharmacodynamic) correlations, drug metabolism characterization, and non-clinical safety evaluations were performed using standard methods.
• Tumor growth inhibition and PK/PD studies were carried out using subcutaneous and orthotopic xenografts in syngeneic and nude mice with the National Cancer Institute (NCI) guidelines and the Guide for Laboratory Animal Care and Use.
• R001 and R002 were compared to two clinically approved, commercially available multikinase inhibitors (MKIs) with anti-RET activity, in vitro and in vivo.

Results

X-ray crystal structures of RET and KDR

X-ray crystal structures of RET (left) and KDR (VEGFR2→right). Potent KDR inhibition is a common feature of multikinase inhibitors (MKIs) that target RET and a primary cause of their dose-limiting toxicities. The C-helix of each enzyme is highlighted in yellow.

100-fold selectivity for KDR (VEGFR2) can be achieved without selective gatekeeper mutagenesis.

Inhibitors are selectively cytotoxic to RET-mutant cells

In vitro parameters for compounds R001, R002 and two clinically approved MKIs. Selectivity is shown as a ratio of EC50s for the indicated kinase compared to wild-type RET. Lower ≥100-fold selectivity was observed for >95% of 228 purified kinase domains in radiometric kinase assays, including additional kinases (shown) commonly inhibited by MKIs. % inhibition compared to control is shown. A value ≤50 is equivalent to ≥100-fold selectivity.

High potency and selectivity for RET can be achieved

In vitro assay of protein lysates. Compounds (colored circles) shifted up and to the left more potent against KIF5B-RET than KDR. Right: Similar analysis for wild-type RET and V804M RET in enzyme assays at 1nM ATP. R001 and R002 (lower left quadrant) maintain potency against V804M RET within 7- and 2-fold of wild-type RET, respectively, while two commercially available MKIs with anti-RET activity demonstrate loss of inhibitory activity.

Dose-dependent inhibition of phospho-RET and tumor growth in NIH3T3-KIF5B RET allografts

Upper In vivo parameters for compounds R001, R002 and two clinically approved MKIs. % inhibition compared to control is shown. A value ≤50 is equivalent to ≥100-fold selectivity.

Summary

We have identified a series of potent and selective RET inhibitors with high oral bioavailability and favorable PK properties in animals. Several demonstrated potent activity against wild-type RET and other kinases with clinical activity and selectivity against RET and its related kinases. In an NIH3T3-KIF5B RET allograft model, Compound R002 effectively inhibited phospho-RET and caused dramatic tumor growth inhibition with minimal toxicity. The identification of potent and selective RET inhibitors with significant in vivo activity and minimal toxicity may overcome the limitations of currently available inhibitors with anti-RET activity.