Antitumor Activity of RXDX-105 in Multiple Cancer Types with RET Rearrangements or Mutations

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Abstract

Purpose: While multikinase inhibitors with RET activity are active in RET-rearranged thyroid and lung cancers, objective response rates are relatively low and toxicity can be substantial. The development of novel RET inhibitors with improved potency and/or reduced toxicity is thus an unmet need. RXDX-105 is a small molecule kinase inhibitor that potently inhibits RET. The purpose of the preclinical and clinical studies was to evaluate the potential of RXDX-105 as an effective therapy for cancers driven by RET alterations.

Experimental design: The RET-inhibitory activity of RXDX-105 was assessed by biochemical and cellular assays, followed by in vivo tumor growth inhibition studies in cell line- and patient-derived xenograft models. Antitumor activity in patients was assessed by imaging and Response Evaluation Criteria in Solid Tumors (RECIST).

Results: Biochemically, RXDX-105 inhibited wild-type RET, CCDC6-RET, NCOA4-RET, PRKAR1A-RET, and RET M918T with low to subnanomolar activity while sparing VEGFR2/KDR and VEGFR1/FLT. RXDX-105 treatment resulted in dose-dependent inhibition of proliferation of CCDC6-RET-rearranged and RET C634W-mutant cell lines and inhibition of downstream signaling pathways. Significant tumor growth inhibition in CCDC6-RET, NCOA4-RET, and KIF5B-RET–containing xenografts was observed, with the concomitant inhibition of p-ERK, p-AKT, and p-PLCγ. Additionally, a patient with advanced RET-rearranged lung cancer had a rapid and sustained response to RXDX-105 in both intracranial and extracranial disease.

Conclusions: These data support the inclusion of patients bearing RET alterations in ongoing and future molecularly enriched clinical trials to explore RXDX-105 efficacy across a variety of tumor types. Clin Cancer Res; 23(12): 2981–90. ©2016 AACR.

Introduction

The rearranged during transfection (RET) gene is an established proto-oncogene. It encodes a single-pass transmembrane receptor tyrosine kinase that is required for the development, maturation, and maintenance of a number of tissues and cell types (1). Under normal conditions, the binding of glial cell line-derived neurotrophic factor (GDNF) family ligands to RET on the cell surface (2) leads to dimerization and auto-phosphorylation of intracellular tyrosine residues. This, in turn, results in the activation of downstream RAS–MAPK, PI3K–AKT, and phospholipase Cγ (PLCγ) pathways (3), and increased cell survival and proliferation.

Ablation of RET signaling can occur via a variety of mechanisms. Germline gain-of-function RET mutations are identified in patients with multiple endocrine neoplasia type 2 (MEN2) and familial medullary thyroid cancer (MTC). In addition, somatic RET mutations are found in the majority of sporadic MTC (4). Such mutations lead to constitutive receptor activation and are found in either the extracellular or intracellular kinase domains of the protein. Examples of activating RET mutations include C634W, M918T, and the gatekeeper mutations, V804L and V804M.

In contrast, recurrent RET gene rearrangements, resulting in the expression of oncogenic RET fusion proteins, have been detected in papillary thyroid carcinoma (PTC; ref. 5) and other tumor types, including non–small cell lung cancer (NSCLC; refs. 6–8) and colorectal cancer (CRC; refs. 9, 10). A variety of upstream partners (7, 8) provide coiled-coil domains that cause ligand-independent dimerization and constitutive activation of the RET kinase (6). These fusion oncoproteins are transforming in vitro and in vivo in engineered Ba/F3 cells and NIH-3T3 cells (6, 7, 11, 12), and in genetically engineered mouse models (GEMM) in which KIF5B-RET was expressed in lung epithelial cells (13, 14).

RET inhibitors are active in patients with RET-rearranged or RET-mutant solid tumors (15). In thyroid cancers, for example, the multikinase inhibitors (MKI) cabozantinib, vandetanib, and lenvatinib have been approved for treatment based on improvements in response and progression-free survival (PFS; ref. 16). The efficacy of these MKIs is believed to be driven by RET inhibition, but the involvement of other mechanism of action such as antiangiogenesis cannot be excluded. The activity of cabozantinib...
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Translational Relevance

To address the relatively low and variable objective response rates and substantial toxicity associated with current therapeutic agents, we are developing a novel RET inhibitor, RXDX-105, for the treatment of molecularly selected patients with RET alterations. Preclinically, RXDX-105 is a potent inhibitor against RET, RET fusions and certain activating RET mutations in the biochemical and cellular assays. In a panel of RET-rearranged, patient-derived xenograft (PDx) models representing different histologies and different fusion partners, RXDX-105 inhibited tumor growth at clinically achievable concentrations. The preclinical observations were then confirmed clinically by a RECIST partial response (PR) of both intracranial and extracranial disease in a RET-rearranged lung cancer patient treated with RXDX-105. Together, these data support the inclusion of patients bearing RET alterations in ongoing and future molecularly enriched clinical trials to explore RXDX-105 efficacy across a variety of tumor types.

Materials and Methods

Compound and cell lines

Good manufacturing practice (GMP)-quality RXDX-105, formerly named CEP-32496, was synthesized at Ignyta. The human lung adenocarcinoma cell line LC-2/2ad (Cat. No. 94072247) and the medullary thyroid cancer cell line TT (CRL-1803) were obtained from Sigma-Aldrich and the American Type Culture Collection (ATCC), respectively. Both cell lines were obtained directly, within 6 months of the studies, from the respective cell banks with certificate of short tandem repeat (STR) authentication. Cells were propagated in F-12K or RPMI medium supplemented with 10% (vol/vol) FBS as recommended. All cell lines were maintained in a humidified incubator at 37°C in a 5% CO2 environment. The HBEC3KT-RET cell line was generated by expressing CCDC6-RET and a dominant-negative p53 (C-terminus region of wild-type p53; ref. 25) in HBEC3-KT cells (human bronchial epithelial cells immortalized with CDK4 and hTERT; ref. 26).

3-Dimensional modeling of RXDX-105 binding to RET

The x-ray co-crystal structure of RXDX-105 in complex with RET has not been determined. However, a similar analogue in the same series of RXDX-105 was successfully co-crystallized with RET. This complex structure was determined with a resolution of 1.7 Å and was used for modeling. Glide, as implemented in Schrodinger’s modeling suite, was used for docking of the RXDX-105 analogue into the RET binding site. The docked poses were subjected to further optimization with Prime MMGBSA.

Biochemical kinase assay

RXDX-105 biochemical IC50 values were determined using vendor protocols at the Ks level of ATP by the Reaction Biology Corporation using the radioactive HotSpot assay platform.

Western blot analysis and phospho-protein profiling

Cells were seeded at a density of 5 × 10^5 cells per well in 6-well plates and cultured for 24 hours. The cells were then treated with 50 to 5,000 nmol/L of the indicated compounds for 2 hours and harvested/lysed in 1x RIPA buffer containing Halt protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific). Lysates were quantified using the Pierce 660 nmol/L protein assay kit (Thermo Fisher Scientific). Twenty-five to 30 μg of protein was resolved on 8% denaturing SDS-polyacrylamide gels, transferred to PVDF membranes, and blotted with indicated primary antibodies followed by HRP-conjugated secondary antibodies (LI-COR Biotechnology). Bands were detected by enhanced chemiluminescence (GE Healthcare). To make lysate from xenografted tumor, frozen tumor tissue was weighed, and approximately 100 mg tumor tissue was placed in 200 μL RIPA buffer. The tissue was then homogenized in RIPA buffer using FastPrep-24 5G (MP Bio) according to the manufacturer’s protocol. After homogenization, the samples were centrifuged at 14,000 × g for 10 minutes at 4°C. The supernatant was isolated, protein was quantitated, and 30 μg/lane protein was separated by 4% to 20% SDS-PAGE for immunoblotting. All primary antibodies used in these studies were obtained from Cell Signalling Technology and include Phospho-RET (Tyr905; #3221), RET (#3220), phospho-MEK1/2 (Ser217/221; #9154), MEK1/2 (#9126), Phospho-ERK (T202/Y204; #9101), ERK (#4695), Phospho-AKT (S473; #4060), AKT (#4691), Phospho-PLCg (Y783; #2821), PLCg (#5690), and β-Actin (#3700).

For phospho-protein profiling, 5 × 10^6 cells were plated in 10-cm dishes, then deprived of serum for 24 hours. Cells were then treated with 1 μmol/L RXDX-105 for 30 minutes. Protein phosphorylation was determined using a phosphokinase profiling array obtained from R&D Systems, according to the manufacturer’s instructions.

Cell viability assays

LC-2/ad and TT cells were seeded at a density of 5,000 cells per well in 96-well plates in medium containing 10% (vol/vol) FBS. The following day, cells were serum-starved in 0.5% FBS-containing media for 24 hours and then treated with the indicated
RXDX-105 was carried out at Crown Biosciences. Model CR1520 and cleaved PARP (Cell Signaling Technology #9541) were compounds for 24 hours. Cell lysate was prepared in RIPA lysis buffer. The cleaved Caspase-3 (Cell Signaling Technology #9661) and cleaved PARP (Cell Signaling Technology #9541) were measured by standard Western blot method.

**Apoptosis assay**

The cells were split into 10-cm dishes. Twenty-four hours later, the cells were treated with different concentrations of indicated compounds for 24 hours. Cell lystate was prepared in RIPA lysis buffer. The cleaved Caspase-3 (Cell Signaling Technology #9661) and cleaved PARP (Cell Signaling Technology #9541) were measured by standard Western blot method.

**Patient- and cell line–derived subcutaneous xenograft models**

Studies using colorectal cancer xenograft models CR1520 and CR2518 were carried out at Crown Biosciences. Model CR1520 contains a NCOA4-RET fusion, while model CR2518 contains a CCDC6-RET fusion. No other activating mutations were detected in KRAS, EGFR, PRKCA, AKT, BRAF, ERK, TP53, PTEF, or CTNNB1 in these models. To generate study cohorts, tumor fragments from stock mice or cryopreserved stock were inoculated into the right flank of female BALB/c nude mice. Mice were randomly allocated to treatment groups (6 per group) according to their tumor volume when the average tumor size reached about 150 to 200 mm$^3$. The test articles were formulated in 22% 2-hydroxypropyl-beta-cyclodextrin and administered to the tumor-bearing mice for 14 consecutive days (2 weeks) at 10 mg/kg and 30 mg/kg, twice daily (BID). An additional group of 60 mg/kg, once daily (QD) was included in the CR2518 study. Tumor size and body weight were monitored at least 2 times a week.

Additional in vivo studies using a low-passage PDX model of NSCLC harboring KIF5B-RET fusion were carried out at Champions Oncology. Briefly, models CTG-0838 and CTG-1048 were expanded in nu/nu mice to establish study cohorts. Animals were then randomized into treatment groups based on tumor size, and dosing was initiated once tumors reached a volume that fell within the range of 150 to 300 mm$^3$. The test articles were formulated in 22% 2-hydroxypropyl-beta-cyclodextrin and administered to the tumor-bearing mice for 4 consecutive weeks at 30 mg/kg, BID. Tumor size and body weight were monitored at least 2 times a week.

For the HBECK3T-RET xenograft experiments, cell line xenograft tumors were transplanted from seed mice (nu/nu) to experimental animals as a single subcutaneous tumor. When tumors reached 100 to 150 mm$^3$, mice were randomly assigned to treatment groups (5 mice per group). RXDX-105 was administered by oral gavage at 50 mg/kg and 100 mg/kg QD, with a total of 5 treatments given in a 7-day period. Tumor size and body weight were monitored at least two times a week.

Data were expressed as mean ± SEM unless indicated otherwise. Statistical significance was determined by analysis of variance (ANOVA) using Dunnett multiple-comparison posttest with GraphPad Prism software unless otherwise noted.

**Results**

**RXDX-105 is a potent inhibitor of RET**

The chemical synthesis and characterization of RXDX-105 has been described previously (22, 27, 28). Its structure is depicted in Fig. 1A. Although RXDX-105 was initially identified as an inhibitor of wild-type and V600E-mutated BRAF (28), the kinase profiling of RXDX-105 also indicated potent inhibitory activity against RET (22). As a follow up to these data, we determined the biochemical IC$_{50}$ values against wild-type RET, as well as a select panel of RET fusions and mutations by a cell-free, radioactive kinase assay platform, performed in multiple repeats. Biochemically, RXDX-105 is a potent wild-type RET inhibitor, with an IC$_{50}$ of 0.33 nmol/L. RXDX-105 has similar activity against RET fusions tested that contain wild-type kinase domain, including CCDC6-RET (IC$_{50}$ = 0.33 nmol/L), NCOA4-RET (IC$_{50}$ = 0.41 nmol/L), and PRKAR1A-RET (IC$_{50}$ = 0.81 nmol/L). The drug is active against RET M918T (IC$_{50}$ = 4.34 nmol/L), but displayed reduced activity against the gatekeeper mutations RET V804L (IC$_{50}$ = 319 nmol/L) and RET V804M (IC$_{50}$ = 266 nmol/L; ref. 14) in the biochemical assay. In the same assay, the IC$_{50}$ values of RXDX-105 against VEGFR1/FLT and VEGFR2/KDR are 140.60 nmol/L and 257.60 nmol/L, respectively. The relative selectivity between RET wild-type and VEGFR2/KDR is about 800-fold.

**RXDX-105 is predicted to bind to the DFG-out conformation of RET**

An analogue of RXDX-105 in the same series was successfully co-crystallized with RET. This complex structure was determined with a resolution of 1.7 Å and was used for modeling. Glide was used for docking of RXDX-105 into the RET binding site and docked poses were subjected to further optimization with Prime MMGBSA. In this model, the RXDX-105 analogue was found to bind to the DFG-out, inactive conformation of RET. The molecule was buried deeply in the ATP binding site as well as a back pocket, interacting extensively with residues in the ATP site, DFG loop, sC-helix (Fig. 1B and C). Specifically, the drug hydrogen bonds with the NH of Ala 807 in the hinge region and Ser 891 in the activation loop, as well as side-chain carboxyl group of Glu 775 in the sC-Helix. In addition, the molecule was predicted to make extensive hydrophobic interactions with the gatekeeper residue Val 804, Phe 893 of the DFG loop, as well as side-chains of Val 778, Leu 779, Val 782, Val 787, Leu 865, and Leu 870 in the back pocket.
RXDX-105 is active in vitro against RET-rearranged and RET-mutant models

We first characterized the ability of RXDX-105 to inhibit RET activation in two independent cellular models harboring distinct RET alterations. The LC-2/ad lung adenocarcinoma and the TT medullary thyroid cancer cell lines harbor a CCDC6-RET gene fusion and RET C634W activating point mutation, respectively. Cells were incubated with 50 nmol/L to 5 μmol/L RXDX-105 for 2 hours, then lysed in RIPA buffer containing phosphatase and protease inhibitors. The relative protein and phospho-protein levels were qualitatively determined by Western blot analysis. Treatment with RXDX-105 led to robust decrease in phosphorylation of RET and PLCγ in both cellular models (Fig. 2). In LC-2/ad cells, RXDX-105 activity against RET and PLCγ was comparable with, or slightly higher than that achieved with cabozantinib and alectinib, two clinically approved multikinase inhibitors (for thyroid cancer and ALK-rearranged lung cancer, respectively) that have RET activity (Fig. 2A). However, while cabozantinib was able to decrease phospho-ERK and phospho-MEK at as low as 50 nmol/L, neither RXDX-105 nor alectinib treatment showed detectable change in ERK and MEK phosphorylation. In TT cells, however, RXDX-105 outperformed cabozantinib and alectinib in decreasing phospho-RET, phospho-PLCγ, phospho-ERK, and phospho-MEK (Fig. 2B). Despite the difference in MARK pathway response between LC-2/ad and TT cells, RXDX-105 effectively inhibited both cell lines in the antiproliferative assay. LC-2/ad and TT cells were treated with various doses of RXDX-105, and cell viability was measured after three (LC-2/ad) or four days (TT). In LC-2/ad cells, RXDX-105 (IC50 = 40 nmol/L) out-performed both cabozantinib (IC50 = 98 nmol/L) and alectinib (IC50 = 204 nmol/L). Similarly, RXDX-105 exhibited more potent activity in TT cells, with an IC50 of 11 nmol/L, compared with 77 nmol/L for cabozantinib and 90 nmol/L for alectinib. To further characterize the mechanism of inhibition, apoptosis was measured by the amount of cleaved caspase-3 and cleaved PARP proteins after 24-hour incubation with RXDX-105, cabozantinib, and alectinib. While in LC-2/ad cells (Fig. 2C), all three compounds induced apoptosis at 24-hour time point, no cleavage of caspase-3 and PARP was seen in the TT cells, except for the higher doses of alectinib (Fig. 2D). The reason for the observed difference in apoptosis upon treatment is unclear.

We next examined if treatment with RXDX-105 could pharmacodynamically and functionally inhibit the proliferation of an engineered RET-dependent cell line, HBEC3KT-RET, derived by expressing CCDC6-RET and a dominant-negative p53 mutant in HBEC3-KT cells, human bronchial epithelial cells immortalized with CDK4 and hTERT (25). Treatment with RXDX-105 for 30 minutes inhibited the phosphorylation of RET, AKT, and ERK (Supplementary Fig. S1A). We then extended the study by looking at the effect of RXDX-105 on the phosphorylation of a larger panel on kinases using a phosphokinase profiling array (R&D Systems). Treatment of serum-deprived cells with 1 μmol/L RXDX-105 caused a robust reduction in phosphorylation of AKT, ERK1/2, STAT1, and WNK1 in HBEC3KT-RET cells (Supplementary

Figure 1.
Fig. S1B). Concurrent with the pathway inhibition, a growth-inhibitory effect ($IC_{50} = 0.44 \mu m/L$) was observed via an alamar blue cell viability assay in these cells treated with RXDX-105 for 96 hours (Supplementary Fig. S1C). Taken together, these results demonstrate that RXDX-105 is a potent RET inhibitor in vitro that is able to abrogate proliferation of RET-dependent human cancer cell lines and engineered bronchial epithelial cells.

RXDX-105 exhibits dose-dependent inhibition of RET-rearranged xenografts

The in vivo efficacy of RXDX-105 was evaluated in four RET fusion–containing xenograft models, including the HBEC3KT-RET cell line–derived xenograft model, one NSCLC PDX model (CTG-0838, Champions Oncology), and two colorectal cancer (CRC) PDX models (CR2518 and CR 1520, Crown Biosciences).

We first evaluated RXDX-105 in the HBEC3KT-RET xenograft model driven by CCDC6-RET. Treatment of HBEC3KT-RET xenografts with 50 mg/kg or 100 mg/kg BID RXDX-105 resulted in a significant reduction in tumor growth (Supplementary Fig. S2A). At the dose of 100 mg/kg, all tumors shrank more than 30% in size by the end of the study (day 7). The average tumor regression was approximately 50% (Supplementary Fig. S2A). Based on body weight measurement, both 50 mg/kg and 100 mg/kg treatments were well tolerated under the regimen of once daily for
5 consecutive days followed by a 2-day dosing break per 7-day cycle (Supplementary Fig. S2C).

Furthermore, we identified low-passage NSCLC PDX models CTG-0838 and CTG-1048, available at Champions Oncology, both of which contain the KIF5B-RET fusion, the most common RET gene rearrangement observed in lung cancer. Treatment of CTG-0838 with RXDX-105 at 30 mg/kg BID resulted in significant tumor growth inhibition (Fig. 3A), accompanied by inhibition of phospho- and total RET, ERK, PLCγ, and AKT (Fig. 3B). Similarly, treatment of CTG-1048 with RXDX-105 resulted in significant regression in all tumor-bearing mice (Fig. 3C).

In addition to NSCLC, we further tested RXDX-105 in two CRC PDX models harboring distinct RET rearrangements. Based on sequencing data provided by Crown Biosciences and confirmed in-house at Ignyta, model CR2518 possesses a CCDC6-RET gene fusion, joining exon 1 of CCDC6 to exon 12 of RET; and model CR1520 possesses a NCOA4-RET gene fusion, joining exon 6 of NCOA4 to exon 12 of RET. Both models were dosed with RXDX-105 at 10 and 30 mg/kg BID, and CR2518 was dosed with an additional 60 mg/kg QD dose group. As expected from our in vitro data, treatment with RXDX-105 produced a dose-dependent inhibition of tumor growth, with 30 mg/kg BID inducing tumor regression in all tumors in both models (Figs. 4A and B, 5A and B). In the CR2518 model, treatment with 30 mg/kg BID was equivalent to the 60 mg/kg QD dose group (Fig. 4A and B). Importantly, in these studies, RXDX-105 was well tolerated in all dose groups and did not result in any significant body weight loss over the course of the studies (Fig. 4C; Supplementary Fig. S2C).

RXDX-105 treatment results in a rapid and sustained response in a patient with advanced RET-rearranged lung cancer with brain metastasis

A 33-year-old female never smoker presented with worsening dyspnea in February of 2016. Workup including computed

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Figure 3. In vivo efficacy of RXDX-105 in a PDX model of NSCLC harboring KIF5B-RET fusion. A, Tumor growth inhibition of CTG-0838 by RXDX-105 orally administered BID at 30 mg/kg. Tumor sizes are presented as average ± SEM (**, P < 0.01). B, At the end of the study (day 27), tumor samples randomly selected from two mice per group were collected two and 12 hours after the final treatment. Western blot was performed using antibodies against phospho- and total RET, ERK, PLCγ, and AKT. C, Tumor growth inhibition of CTG-1048 by RXDX-105. Tumor sizes are presented as average ± SEM (**, P < 0.01).
in vivo imaging (MRI) of the brain identified adenopathy, and bony metastases. Magnetic resonance imaging revealed a hypermetabolic right perihilar mass, mediastinal lymphadenopathy, and consistent with a lung adenocarcinoma. Immunohistochemistry was positive for TTF-1 and signet ring features. Histological analysis revealed adenocarcinoma with hybridization (FISH) testing. Split red and green signals were detected in 60% of the nuclei scored. Targeted next-generation sequencing on an Ion Torrent PGM instrument did not reveal any mutations with known significance. FISH testing for TP53 underlies the rationale to pharmacologically target their vulnerabilities.

A RET rearrangement was detected by dual-color break-apart fluorescence in situ hybridization (FISH) testing. Split red and green signals were detected in 60% of the nuclei scored. Targeted next-generation sequencing on an Ion Torrent PGM instrument (Thermo Fisher Scientific) for specific mutations in 50 genes including EGFR, ERBB2, BRAF, KRAS, MET, PTEN, PIK3CA, and TP53 did not reveal any mutations with known significance. FISH testing for ALK and ROS1 did not reveal recurrent gene rearrangements involving these genes.

The patient was enrolled onto the phase Ib portion of an ongoing phase I/ib clinical trial of RXDX-105 (NCT01877811). The drug was administered at 350 mg daily. Of note, she had not received any prior chemotherapy, immune checkpoint inhibition, or radiotherapy for her cancer. A rapid partial response was achieved after 3 weeks of therapy with RXDX-105, with 48% shrinkage of the patient’s target lesions by RECIST v1.1. This response was confirmed at 8 weeks of therapy with note of further reduction in tumor burden. Repeat imaging of the brain likewise revealed almost complete resolution of the previously noted three subcentimeter asymptomatic brain metastases. This radiologic response was accompanied by a significant improvement in the patient’s dyspnea. She currently remains on RXDX-105 with continued disease control and a best objective response of a 78% decrease in tumor burden from baseline (Fig. 6). She did develop a grade 1 rash that resulted in dose reduction of the drug to 200 mg daily with subsequent improvement. The patient remains on treatment with RXDX-105 at 6.7 months with no major issues with treatment tolerance.

Discussion

In recent years, the extensive genomic characterization of cancers has led to the discovery of molecular alterations involved in tumorigenesis, metastasis, and drug resistance. The dependency of the tumors on these driver events in various tumor histologies underlies the rationale to pharmacologically target their vulnerabilities using specific inhibitors (29). RET rearrangements are present in approximately 1% to 2% of lung adenocarcinomas and 20% to 40% of sporadic papillary thyroid cancers and tend to occur in a mutually exclusive fashion with other known driver alterations (30, 31). In addition, RET rearrangements have been identified in other cancer types such as CRC (9), breast cancer (32), Spitz tumors (33), and chronic myelomonocytic leukemia (34). Furthermore, RET mutations are known drivers of oncogenesis in tumors such as medullary thyroid cancer.

RXDX-105 is an orally available, VEGFR-sparing, multikinase inhibitor that is potent against RET in a wide range of in vitro and in vivo models of RET-rearranged and RET-mutant tumors. The drug is predicted to bind to the DFG-out conformation of RET. Biochemically, RXDX-105 potently inhibited wild-type RET and RET fusion proteins with IC50 values below 1 nmol/l. In addition, RXDX-105 was able to inhibit RET M918T, an activating mutation found in the majority of cases of multiple endocrine neoplasia type 2B (35). However, point mutations at V804 site, V804L, and V804M, could render the tumor less sensitive to RXDX-105 treatment based on the biochemical activity, but whether this is...
true in the clinic requires further clinical evidence (14). Inhibition of RET was confirmed in cells by measuring RET pathway phosphorylation, and this correlated with inhibition of cell proliferation. Furthermore, in multiple cell line- and patient-derived NSCLC and CRC xenograft models harboring KIF5B-RET, CCDC6-RET, and NCOA4-RET, RXDX-105 was able to stabilize or cause tumor regression at clinically achievable doses (36). These preclinical studies support the inclusion of patients bearing RET alterations in future clinical trials exploring RXDX-105 efficacy across a variety of tumor types.

While other multikinase inhibitors with RET activity are either approved or currently in clinical development for thyroid and lung cancers, response rates are low even in genomically enriched subgroups, and toxicity can be significant. In RET-rearranged lung cancers, the overall response rate with cabozantinib in a phase II trial was 28% (17). While this activity is comparable with
RXDX-105 Is Efficacious in RET-Driven Tumors

The drug displayed potent and dose-dependent in vivo antitumor activity with significant tumor regression in cell line- and patient-derived xenograft models harboring RET rearrangements. A rapid and sustained response to therapy was observed in a patient with an advanced RET-rearranged lung cancer. These data support the inclusion of patients whose tumors harbor RET fusions or mutations in ongoing molecularly enriched clinical trials to explore the efficacy of RXDX-105 across a variety of tumor types.

Disclosure of Potential Conflicts of Interest

G.G. Li has ownership interest (including patents) in Ignyta. G.J. Riely is a consultant/advisory board member for Genentech/Roche and Novartis, and reports receiving commercial research support from Ariad, Genentech/Roche, Millennium, Novartis, and Pfizer. A. Drilon reports receiving speaker fees from Ignyta. No potential conflicts of interest were disclosed by the other authors.

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