ABSTRACT

The discovery of RET fusions in lung cancers has uncovered a new therapeutic target for patients whose tumors harbor these changes. In an unselected population of non–small cell lung carcinomas (NSCLCs), RET fusions are present in 1% to 2% of cases. This incidence increases substantially, however, in never-smokers with lung adenocarcinomas that lack other known driver oncogenes. Although preclinical data provide experimental support for the use of RET inhibitors in the treatment of RET fusion-positive tumors, clinical data on response are lacking. We report preliminary data for the first three patients treated with the RET inhibitor cabozantinib on a prospective phase II trial for patients with RET fusion-positive NSCLCs (NCT01639508). Confirmed partial responses were observed in 2 patients, including one harboring a novel TRIM33–RET fusion. A third patient with a KIF5B–RET fusion has had prolonged stable disease approaching 8 months (31 weeks). All three patients remain progression-free on treatment.

SIGNIFICANCE: Driver oncogene discovery in lung cancers has dramatically changed today’s therapeutic landscape. This report of the activity of cabozantinib in RET fusion-positive disease provides early clinical validation of RET fusions as drivers in lung cancers and suggests that RET inhibition may represent a new treatment paradigm in this molecular cohort.

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INTRODUCTION

Recurrent gene fusions have emerged as important oncogenic drivers of a variety of hematologic and solid tumor malignancies (1). Among non–small cell lung carcinomas (NSCLCs), rearrangements in ALK and ROSI are present in at least 5% of lung adenocarcinomas (2, 3). The corresponding fusion proteins contain an intact tyrosine kinase domain fused to upstream partners that often provide dimerization domains (4, 5). Constitutive kinase activity results in activation of downstream pathways involved in tumor cell growth and proliferation. ALK and ROSI fusions are nonoverlapping with other known drivers in lung cancer, such as mutations in KRAS and EGFR, and are more commonly found in adenocarcinomas from never-smokers (2, 6). Their role as potent oncogenic drivers is underscored by the dramatic clinical responses seen with crizotinib, a tyrosine kinase inhibitor of ALK and ROSI, in patients who harbor these rearrangements (7, 8).

Activation of RET is a mechanism of oncogenesis in medullary thyroid carcinomas where both germline and sporadic activating somatic mutations are prevalent (9). Gene rearrangements involving RET, on the other hand, have been characterized most extensively in papillary thyroid carcinomas,
particularly those discovered in the wake of significant radiation exposure, such as in survivors of the Chernobyl nuclear disaster. The incidence of RET fusions in papillary thyroid carcinomas increases to 60% to 80% in the latter (10, 11).

Ji and colleagues (12) reported the first case of a RET fusion in lung cancer in 2011. The KIF5B–RET fusion was discovered by whole genome and transcriptome sequencing of tumor tissue from a never-smoker with advanced adenocarcinoma of the lung. Several independent groups have since reported the detection of these fusions, uncovering a new molecular subset of lung cancers sharing remarkably similar features with rearrangements of ALK and ROS1 (13–16). Oncogenic potential has been shown in vitro in transfected NIH3T3 and Ba/F3 cells, and RET inhibition with vandetanib, sunitinib, and sorafenib resulted in loss of cell viability and abrogation of the transformed phenotype, suggesting that RET might be a druggable target (14–16). However, data establishing the use of RET inhibitors in the clinic are lacking.

RESULTS

Given the increased frequency of RET fusions in tumors from never-smokers and their mutual exclusivity with known driver oncogenes (15), we focused on screening an enriched cohort of never-smokers (<100 lifetime cigarettes) with advanced “pan-negative” nonsquamous NSCLCs for the detection of these fusions, uncovering a new molecular subset of lung cancers sharing remarkably similar features with rearrangements of ALK and ROS1.

A total of 31 patients with pan-negative lung adenocarcinomas were prospectively identified after extensive genotyping. RET fusions were found in 5 of 31 patients (16%; 95% confidence interval, 3%–29%) over the course of 10 months. No distinct histologic features were shared between the 5 cases (adenocarcinoma morphology varied: 1 patient with papillary features, 1 with solid morphology, 1 with predominantly papillary features but with solid and lepidic components, 1 with micropapillary and solid morphology, and 1 with poorly differentiated histology). Sites of metastases varied significantly as well. Average and median overall survival from diagnosis for these patients were 30 and 27 months, respectively (with 4 of 5 patients currently alive). Within the limits of a small series, these outcomes were more favorable than the median survival of 12 months of metastatic unselected patients with NSCLC and closer to those seen in EGFR-mutant patients, which range from 20 to 30 months across several large randomized studies (17).

Screening was conducted to determine eligibility for a prospective, single-institution, open-label, phase II study of cabozantinib (XL-184) for RET fusion-positive lung carcinomas initiated in July 2012 (ClinicalTrials.gov number NCT01639508). Cabozantinib, a multi-tyrosine kinase inhibitor and potent inhibitor of RET, was chosen on the basis of the observation that the drug was most effective at inhibiting proliferation in a CCDC6–RET (RET/PTC1) fusion-positive papillary thyroid cancer cell line (IC50, 0.06 μmol/L) compared with vandetanib, sunitinib, and axitinib (18). Of the 5 patients who tested positive for a RET fusion, 1 was ineligible for study participation due to a declining performance status and eventually passed away. One patient only recently tested positive and is to be offered study enrollment. The 3 remaining patients were eligible for treatment and subsequently enrolled in this protocol. Baseline burden of disease was low for all 3 cases.

A novel TRIM33–RET fusion was discovered in a 41-year-old Caucasian female never-smoker with no history of radiation exposure who presented in June 2010 with decreased visual acuity in the right eye. Retinal metastases were noted on ophthalmologic evaluation. In addition, she was found to have a left lower lobe mass and metastatic disease to the pleura and left-sided axillary and supraclavicular lymph nodes. No thyroid masses were noted on computed tomography (CT) or positron emission tomography imaging. A biopsy of a supraclavicular node revealed metastatic adenocarcinoma with papillary morphology (Fig. 1A). Immunohistochemical stains were positive for TTF-1 and napsin-A and consistent with a lung primary.

A RET fusion was present by FISH (Fig. 1B) but negative for KIF5B–RET. Next-generation sequencing showed a TRIM33–RET fusion (Fig. 1C) involving exon 14 of TRIM33 and RET exon 12, which is in-frame. No evidence of MET amplification or mutation was found.

The patient was enrolled in our phase II study of cabozantinib after progression on 2 prior lines of therapy. Cycle 1 toxicities included grade 2 dysgeusia and grade 1 mucositis, diarrhea, and fatigue; subclinical hypothyroidism was managed with thyroid hormone replacement. Follow-up imaging conducted after 4 and 12 weeks of therapy revealed a confirmed partial response with a 66% decrease in measurable disease in the lungs and pleura by Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 (Fig. 2A). A follow-up ophthalmologic examination revealed partial regression of the patient’s bilateral retinal metastases along with resolution of episodic mild blurring of vision. Although sclerotic areas of bony metastasis to the upper sacrum and posterior right ilium were not measurable by RECIST, treatment was accompanied by a clinical response to therapy with the disappearance of tumor-related sacral pain. The patient was not previously treated with a bisphosphonate or anti-RANK ligand therapy. She has been on trial now for 5 months (20 weeks) and remains progression-free and on active therapy.

The second patient was a 75-year-old African-American female never-smoker who was RET fusion-positive by FISH and reverse transcriptase PCR (RT-PCR) for KIF5B–RET. She was initially treated with sequential chemotherapy and radiation for unresectable stage IIIA (T4N1M0) poorly differentiated lung adenocarcinoma. She was subsequently found to have recurrent, metastatic disease, as evidenced by the development of enlarging bilateral pulmonary nodules in the absence of distant disease. She was treated with cabozantinib on-protocol.

Cycle 1 toxicities included grade 3 fatigue requiring cabozantinib dose reduction to 40 mg/day and grade 1 transaminase elevation. Grade 3 proteinuria was a late toxicity requiring further dose reduction to 20 mg/day. Despite the need for dose reductions, the patient had clinical improvement in cough and shortness of breath and a partial response to therapy at 4 weeks (Fig. 2B). This was confirmed at 12 weeks with a decrease in disease burden by 32% by RECIST v1.1. The patient remains progression-free on therapy at 4 months (16 weeks).

The third patient was a 68-year-old Caucasian female never-smoker positive for a RET fusion by FISH. She initially underwent a right upper lobectomy for a stage I lung
adenocarcinoma. She was thereafter found to have metastatic mixed-subtype adenocarcinoma (predominantly papillary with lepidic and solid patterns) with multiple bilateral pulmonary nodules and no evidence of distant disease. She began treatment with cabozantinib after progression of disease on first-line chemotherapy. Cycle 1 toxicities included grade 3 hypertension requiring dose reduction to 40 mg/day of cabozantinib, grade 2 fatigue, and grade 1 skin toxicity. At 4 weeks on-study, she was noted to have stable disease (Fig. 2C) that has since been maintained clinically and radiographically approaching 8 months (31 weeks) into treatment.

**DISCUSSION**

Over the last 5 years, kinase fusions in lung cancers have drawn much attention as targetable driver events. The efficacy of crizotinib for ALK- and ROS1-rearranged lung cancers highlights how the availability of small molecules with multi-kinase activity has greatly facilitated this effort. Interestingly, while crizotinib began early-phase testing in 2005 as a MET inhibitor, the discovery of EML4-ALK fusions (4, 5) in 2007 heralded the demonstration of the activity of crizotinib in ALK fusion-positive lung cancers and subsequent U.S. Food and Drug Administration (FDA) approval for this indication in 2011 (8). Activity of the drug in ROS1-rearranged lung cancer was reported in early 2012 (2). Despite this progress, the timeline between the discovery of genetic driver alterations and the demonstration of activity and eventual approval of a corresponding targeted agent remains a lengthy process that is typically measured in years. This prospective trial of cabozantinib was initiated in July 2012, within only a few months of the discovery of RET fusions reported in late 2011. This illustrates how a rapid bench-to-bedside process allows for accelerated drug development when coupled with a comprehensive molecular analysis of tumor specimens.

The clinical data presented in this series represent the first reports of response to a RET inhibitor in patients on a prospective, molecularly enriched trial for RET fusion-positive lung cancer.

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**Figure 1.** A, photomicrograph of a supraclavicular lymph node biopsy showing a lung adenocarcinoma with papillary morphology. B, a positive RET FISH break-apart test. Split green and red signals indicate the presence of a RET fusion. Probes were designed as previously published [13]. C, the presumptive (1;10)(p13;q11.2) translocation places TRIM33 exons 1 to 14 upstream of RET exons 12 to 18, generating an in-frame TRIM33-RET fusion gene.
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For both responders in this series, the short time frame of clinical and radiographic improvement relative to drug initiation is comparable with the rapid responses observed with erlotinib and crizotinib in EGFR-mutated and ALK-rearranged lung cancers, respectively. Although these findings are highly encouraging, completion of this trial will provide data on long-term follow-up and response in a larger cohort of individuals and will be informative about the durability and overall efficacy of this approach. Furthermore, taking into account the paradigms of resistance shown in other fusion-positive lung cancers (19), our protocol has recently been amended to include repeat biopsies on progression for the evaluation of potential resistance mechanisms. Cabozantinib is a multi-tyrosine kinase inhibitor with effects on VEGF receptor 2 (VEGFR2) likely explaining the off-target effects of hypertension and proteinuria seen in our patients. These toxicities have been manageable with dose modifications and antihypertensive medication, and all patients continue to both tolerate treatment and maintain their responses or stable disease clinically and radiographically.

The process of identification of patients with RET fusion-positive disease was expedited at our institution by the decision to conduct screening in an enriched cohort of individuals who had already been tested for the presence of other known driver mutations. For both responders in this series, the short time frame of clinical and radiographic improvement relative to drug initiation is comparable with the rapid responses observed with erlotinib and crizotinib in EGFR-mutated and ALK-rearranged lung cancers, respectively. Although these findings are highly encouraging, completion of this trial will provide data on long-term follow-up and response in a larger cohort of individuals and will be informative about the durability and overall efficacy of this approach. Furthermore, taking into account the paradigms of resistance shown in other fusion-positive lung cancers (19), our protocol has recently been amended to include repeat biopsies on progression for the evaluation of potential resistance mechanisms. Cabozantinib is a multi-tyrosine kinase inhibitor with effects on VEGF receptor 2 (VEGFR2) likely explaining the off-target effects of hypertension and proteinuria seen in our patients. These toxicities have been manageable with dose modifications and antihypertensive medication, and all patients continue to both tolerate treatment and maintain their responses or stable disease clinically and radiographically.

The process of identification of patients with RET fusion-positive disease was expedited at our institution by the decision to conduct screening in an enriched cohort of individuals who had already been tested for the presence of other known driver mutations. Although the overall prevalence of RET fusions increases from 1% to 2% in an unselected population of NSCLCs to 6% in patients with tumors that are pan-negative for other known driver mutations (15), our preliminary results show that the rate of RET rearrangements in tumors from pan-negative never-smokers is even higher at 16%. If multiplex genotyping for all known drivers is not feasible, current and future testing for these rearrangements will benefit from focusing on this clinically and molecularly enriched population of individuals.

Wang and colleagues (20) recently published the results of RET fusion gene screening of 936 patients with surgically resected NSCLC. Patients with RET fusion-positive lung adenocarcinomas were more likely to be younger (age ≤ 60 years) never-smokers with more poorly differentiated tumors of the solid subtype. Although ALK immunohistochemistry (IHC) has been shown to be useful in detection of ALK rearrangements (21), Wang and colleagues found no statistical difference in RET IHC staining between RET fusion-positive and -negative lung adenocarcinomas. Our experience [using RET antibodies from Epitomics (14) and Vector Labs (15); Hasanovic and Ladanyi, unpublished data] also has been that RET IHC is not sufficiently reliable at present for diagnostic purposes.

This report also represents the first description of the TRIM33–RET fusion in lung cancer. Like ALK and ROS1 rearrangements, RET fusions occur with different partners. KIF5B is the most common of these and is present in approximately 90% of the rearrangements reported to date, with CCDC6 and NCOA4 accounting for the remaining 10% (12–16, 20). All 3 fusions...
are generated via an inversion of the short and long arms of chromosome 10. TRIM33, also known as RFG7 or TIF1γ, is a member of the transcription intermediary factor 1 family that participates in the control of cellular differentiation (22). TRIM33 has previously been reported as a fusion partner of RET (23).

With TRIM33–RET, the 5′ portion of RET is replaced by a gene encoding a coiled-coil domain, resulting in dimerization and ligand-independent activation of the RET tyrosine kinase. These structural features are also seen in CCDC6–RET, CCDC6–RET, and NCOA4–RET (Fig. 3). The history of never smoking and the absence of concurrent driver abnormalities in our patient is similarly consistent with the profile of patients with RET fusions. The TRIM33–RET fusion is not likely to be unique to this patient, as another TRIM33–RET fusion-positive case has recently been detected in The Cancer Genome Atlas lung adenocarcinoma project (24). The continued identification of RET fusion partners in tumors from patients on this prospective trial should provide preliminary data on the potential heterogeneity of response to RET tyrosine kinase inhibition between molecular subtypes.

In conclusion, our series of treatment responses to cabozantinib in patients with RET fusion-positive tumors provides the first clinical data for a new target and drug treatment paradigm in lung cancers. Cabozantinib administration was feasible and toxicities were manageable. RET fusions represent a new addition to the growing list of actionable drivers in lung cancers and merit continued investigation.

**METHODS**

Genotyping was conducted via a mass spectrometry Sequenom platform for 91 point mutations in EGFR, KRAS, NRAS, BRAF, HER2, PIK3CA, MAP2K1, and AKT, multiplex sizing assays for insertions and deletions in EGFR exons 19 and 20 and HER2 exon 20, and FISH break-apart assays for ALK and ROS1 (25). RET fusion FISH assay was conducted via a dual-probe FISH break-apart test. On the basis of an upper level of split signals for break-apart probes on normal formalin-fixed paraffin-embedded tissue sections of approximately 5%, we set the cutoff for scoring the RET FISH assay as positive at 10% of cells with split signals or isolated 3′ signals (red; ref. 13). KIF5B–RET testing was conducted via RT-PCR. Next-generation sequencing of the entire coding sequence of 182 cancer-related genes plus 37 introns of 14 genes commonly rearranged was conducted in a Clinical Laboratory Amendments-certified laboratory (Foundation Medicine; ref. 15).

For this phase II study of cabozantinib in advanced, RET fusion-positive lung cancers, inclusion criteria are as follows: patients with pathologic or cytologic evidence of NSCLC, clinical stage IV or recurrent/medically inoperable disease, a Karnofsky performance status of more than 70%, a life expectancy of more than 12 weeks, adequate hematologic, renal, and hepatic function, measurable disease, and positive testing for a RET fusion via RT-PCR or FISH.

The primary endpoint of the trial is objective response at 12 weeks via RECIST v1.1 (26). Secondary endpoints include progression-free survival, overall survival, and grade 3 or 4 treatment-related adverse events. Patients receive cabozantinib at 60 mg orally daily in 28-day cycles until disease progression or unacceptable toxicity. Imaging studies are conducted at baseline, 4 weeks, and every 8 weeks thereafter. A Simon two-stage minimax design is used to test the null hypothesis of
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a 10% response rate against the desired alternative of a 30% response rate, with a type I error of 10% and a power of 90%. In the first stage of this study, 16 evaluable patients are to be accrued. If responses are noted in 2 or more patients, 9 additional patients will be enrolled, for a total of 25 evaluable patients. The drug will be deemed worthy of further study if a total of 5 responses are seen in this population.

Disclosure of Potential Conflicts of Interest

D. Lipson is employed as Director of Foundation Medicine and has ownership interest (including patents) in the same. P. Stephens has ownership interest (including patents) in Foundation Medicine. J. Ross is employed as Medical Director of Foundation Medicine, has received a commercial research grant from Foundation Medicine, and has ownership interest (including patents) in the same. V. Miller is employed as Senior Vice President, Clinical Development, at Foundation Medicine and has ownership interest (including patents) in the same. M.G. Kris is a consultant/advisory board member of Pfizer, Inc. No potential conflicts of interest were disclosed by the other authors.

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Response to Cabozantinib in Patients with \textit{RET} Fusion-Positive Lung Adenocarcinomas

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