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Systemic and CNS activity of the RET inhibitor vandetanib combined with the mTOR inhibitor everolimus in *KIF5B-RET* re-arranged Non-Small Cell Lung Cancer with brain metastases

Vivek Subbiah, MD[1], **Jenny Berry, RN, MSN, ANP-BC**[1], **Michael Roxas, MPH**[1], **Nandita Guha-Thakurta, MD**[1], **Ishwaria Mohan Subbiah, MD, MS**[1], **Siraj M. Ali, MD**[2], **Caitlin McMahon, BSc**[2], **Vincent Miller, MD**[2], **Tina Cascone, MD PhD**[1], **Shobha Pai, MS, PA-C**[1], **Zhenya Tang, MD**[1], and **John V. Heymach, MD, PhD**[1]

[1]The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030

[2]Foundation Medicine, Boston MA

Abstract

In-frame fusion KIF5B (the-kinesin-family-5B-gene)-RET transcripts have been characterized in 1–2% of non-small cell lung cancers and are known oncogenic drivers. The RET tyrosine kinase inhibitor, vandetanib, suppresses fusion-induced, anchorage-independent growth activity. *In vitro* studies have shown that vandetanib is a high-affinity substrate of breast cancer resistance protein (Bcrp1/Abcg2) but is not transported by P-glycoprotein (P-gp), limiting its blood-brain barrier penetration. A co-administration strategy to enhance the brain accumulation of vandetanib by modulating P-gp/Abcb1- and Bcrp1/Abcg2-mediated efflux with mTOR inhibitors, specifically everolimus, was shown to increase the blood-brain barrier penetration. We report the first bench to bed-side evidence that RET inhibitor combined with an mTOR inhibitor is active against brain-metastatic RET-rearranged lung cancer and the first evidence of blood-brain barrier penetration. A 74 year old female with progressive adenocarcinoma of the lung (wild-type EGFR and no ALK rearrangement) presented for therapy options. A deletion of 5'RET was revealed by FISH assay, indicating RET-gene rearrangement. Because of progressive disease in the brain, she was enrolled in a clinical trial with vandetanib and everolimus (NCT01582191). Comprehensive genomic profiling revealed fusion of KIF5B (the-kinesin-family-5B-gene) and RET, in addition to AKT2 gene amplification. After 2 cycles of therapy a repeat MRI brain showed a decrease in the intracranial disease burden and PET /CT showed systemic response as well. Interestingly, AKT2 amplification seen is a critical component of the PI3K/mTOR pathway, alterations of which has been associated with both *de novo* and acquired resistance to targeted therapy. The addition of everolimus may have both overcome the AKT2 amplification to produce a response in addition to its direct effects on the RET gene. Our case report forms the first evidence of blood-brain-barrier penetration by vandetanib in combination with everolimus.Further research is required in this setting.

Corresponding author: Vivek Subbiah, MD, Department of Investigational Cancer Therapeutics, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd., FC8.3038, Box 0455, Houston, TX 77030, vsubbiah@mdanderson.org; phone 713-563-0893; fax 713-792-3535.

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Keywords

RET; mTOR; Lung cancer; Brain metastases; Blood brain barrier; RET fusion; AKT; Next generation sequencing; Vandetanib; Everolimus; exceptional responder; outlier responder

Introduction

Discovery of ‘driver oncogenes’ and molecularly targeted therapies with inhibitory drugs targeting the oncogenes have revolutionized the therapeutic landscape of non-small cell lung cancer (NSCLC). Targeted therapeutics are replacing chemotherapy even in the front-line setting. In addition to tumors harboring the EGFR mutations that respond to EGFR tyrosine kinase inhibitors (erlotinib, geftinib)[1] and ALK rearranged tumors that remarkably respond well to ALK inhibitors (crizotinib, ceretinib), recently RET (rearranged during transfection) fusions have been discovered in NSCLC[1, 2]. RET is a receptor tyrosine kinase that is primarily expressed in cells of the nervous system[2, 3]. It has been identified as a proto-oncogene. Recombination of the RET locus with a partner gene has been shown to result in cellular transformation[3]. In the TCGA dataset RET rearrangement was observed in 2 of 513 lung adenocarcinoma cases[4]. Other studies have identified RET rearrangement in 1–2% of cases[2, 5–7] and at an incidence of 6% in non-small cell lung cancer patients lacking other known driver mutations[7]. Multiple activating RET fusions with distinct fusion partners have been described in cancer[4, 8], with the KIF5B-RET and CCDC6-RET fusions being most the common variants in NSCLC. Several multi-kinsae inhibitors with activity against RET have been approved by the US FDA in various indications, including vandetanib, cabozantinib, ponatinib, sunitinib, regorafenib, and sorafenib. Preclinical studies demonstrated that cells transformed by KIF5B-RET are sensitive to treatment with vandetanib, sorafenib, and sunitinib[2, 9, 10]. These agents and other drugs targeting RET are in clinical trials in NSCLC and other solid tumors. Preliminary data from an ongoing Phase 2 study reported a partial response in an NSCLC patient harboring the KIF5B-RET fusion treated with cabozantinib[7]. Another case reported an ongoing 5 month response to vandetanib in a patient with NSCLC harboring a CCDC6-RET fusion[11]. However, a retrospective evaluation of RET biomarker status and outcome to vandetanib monotherapy in four phase III randomized NSCLC trials failed to show any differential advantage in RET fusion patients[12]. In addition the blood brain barrier penetration of vandetanib in RET rearranged lung cancer is not reported. Our case report forms the first bench-to-bedside evidence of blood-brain-barrier penetration by RET inhibitor vandetanib combined with the mTOR inhibitor everolimus.

Case history

A 74-year-old Caucasian female who was a former light smoker presented with worsening dyspnea and weight loss. A chest x-ray revealed a right pleural effusion. Thoracentesis drained 1.8 liters of bloody fluid. An FNA cytologic examination revealed adenocarcinoma of the lung (wild-type EGFR and no ALK rearrangement). An MRI of the brain demonstrated brain metastases, for which she underwent gamma knife radiosurgery. PET/CT revealed the presence of metastatic osseous lesions at T2, L1, and hemipelvis. She

underwent 6 cycles of carboplatin and pemetrexed with zoledronic acid, followed by docetaxel, for 8 cycles. A follow-up MRI of the brain revealed progression of disease, with multiple enhancing lesions throughout the brain parenchyma that were 1 to 6 mm in size. She underwent whole-brain radiation therapy. A re-staging CT chest showed a right soft tissue nodule in the lower lobe that measured 1.7 cm, and loculated right pleural effusion. She presented to our institution for further treatment options. A 50-gene panel based on exome sequencing was negative. A deletion of 5'RET was revealed by FISH assay, indicating RET-gene rearrangement (Figure-1). Because of progressive disease in the brain, she was enrolled in a clinical trial with vandetanib (300 mg Q daily) and everolimus (10 mg Q daily) (NCT01582191). The time interval between the whole brain radiation therapy and re-staging MRI brain was 4.5 months. Fortuitously, comprehensive genomic profiling was also performed in parallel and revealed fusion of KIF5B (the-kinesin-family-5B-gene) and RET (Figure-2), in addition to AKT2 and several gene amplifications (Table 1). The patient tolerated therapy reasonably well, with grade-2 diarrhea that required loperamide. Her performance status and quality of life improved. After 2 cycles of therapy a repeat MRI of the brain showed a decrease in the intracranial disease burden (Figure-3-A–D). A PET showed response in the right hemithorax as well (Figure-3-E,F).

Discussion

We report the first evidence that RET inhibitor-based therapy is active against brain-metastatic RET-rearranged lung cancer and the first evidence of blood-brain barrier penetration. In-frame fusion KIF5B-RET transcripts have been characterized in 1–2% of non-small cell lung cancers [2, 6]. The KIF5B-RET fusion reported here is predicted to activate the RET kinase, resulting in oncogenic transformation[2, 9, 10, 13] The RET tyrosine kinase inhibitor, vandetanib, suppresses fusion-induced, anchorage-independent growth activity; it has been translated to the clinic and has been described in published case reports[14]. Moreover, vandetanib targets RET as well as tumor endothelium[15]. However, single agent vandetanib response in lung cancer has been low [16] and monotherapy with vandetanib failed to show any differential advantage in RET fusion patients in a large retrospective study[12].

In vitro studies have shown that vandetanib is a high-affinity substrate of breast cancer resistance protein (Bcrp1/Abcg2) but is not transported by P-glycoprotein (P-gp), limiting its blood-brain barrier penetration[17]. A co-administration strategy to enhance the brain accumulation of vandetanib by modulating P-gp/Abcb1- and Bcrp1/Abcg2-mediated efflux with mTOR inhibitors, specifically everolimus, was shown to increase the blood-brain barrier penetration[17]. Our case report forms the first bench-to-bedside evidence of blood-brain-barrier penetration by vandetanib in combination with everolimus. Interestingly, *AKT2* amplification seen is a critical component of the PI3K/mTOR pathway, alterations of which has been associated with both *de novo* and acquired resistance to targeted therapy. *AKT2* (also known as PKB-beta) encodes an intracellular serine/threonine kinase that is one of three members of the AKT gene family[18]. Activation of AKT2 has been implicated in multiple malignancies[18]. Alterations in the PI3K/AKT signaling pathway are frequent in lung cancer[19]. In the TCGA datasets, AKT2 amplification was found in 1.3% of lung adenocarcinoma cases and 4.5% of lung squamous cell carcinoma cases[20]. There are no

US FDA approved therapies to address the amplification of AKT2. The mTOR inhibitors everolimus and temsirolimus are US FDA approved for the treatment of other tumor types, and these agents can impact the AKT pathway. Clinical studies have shown some activity of everolimus, either alone or in combination with other therapies, in NSCLC[21, 22].

The addition of everolimus may have both overcome the AKT amplification to produce a response in addition to its direct effects on the RET gene[23, 24]. This leads to the hypothesis that combined RET and mTOR pathway inhibition may overcome the innate and/or acquired resistance to RET-targeted monotherapy, as has been demonstrated in other systems[24–27]. In conclusion, RET inhibitor-based therapy may be active against brain metastatic RET-rearranged lung cancer due to potential blood-brain barrier penetration. Further research is required in this setting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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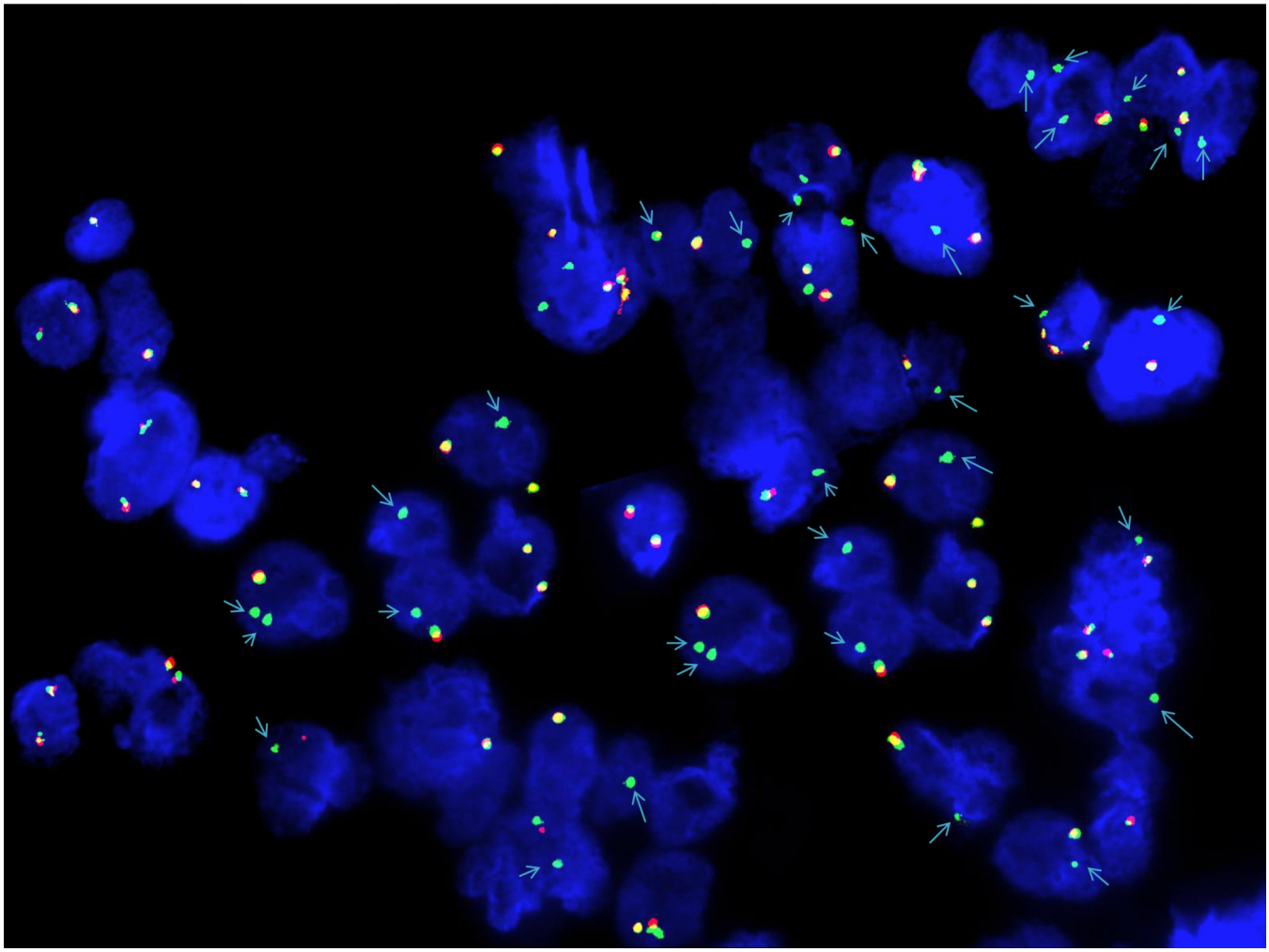

Figure 1.

Fluorescence in situ hybridization (ISH) of paraffin-embedded tissue sections from pleura, right biopsy. The ISH technique was performed using a Clear-View FISH RET dual-color breakapart probe from Cymogen DX. The probe hybridizes to band 10q11.21 (cmyo-orange on the centromeric side and cymo-green on the telomeric side of the RET gene breakpoint). Deletion of 5'-RET (red signal) was observed in 47.0% of the interphases scanned, indicating a RET gene rearrangement.

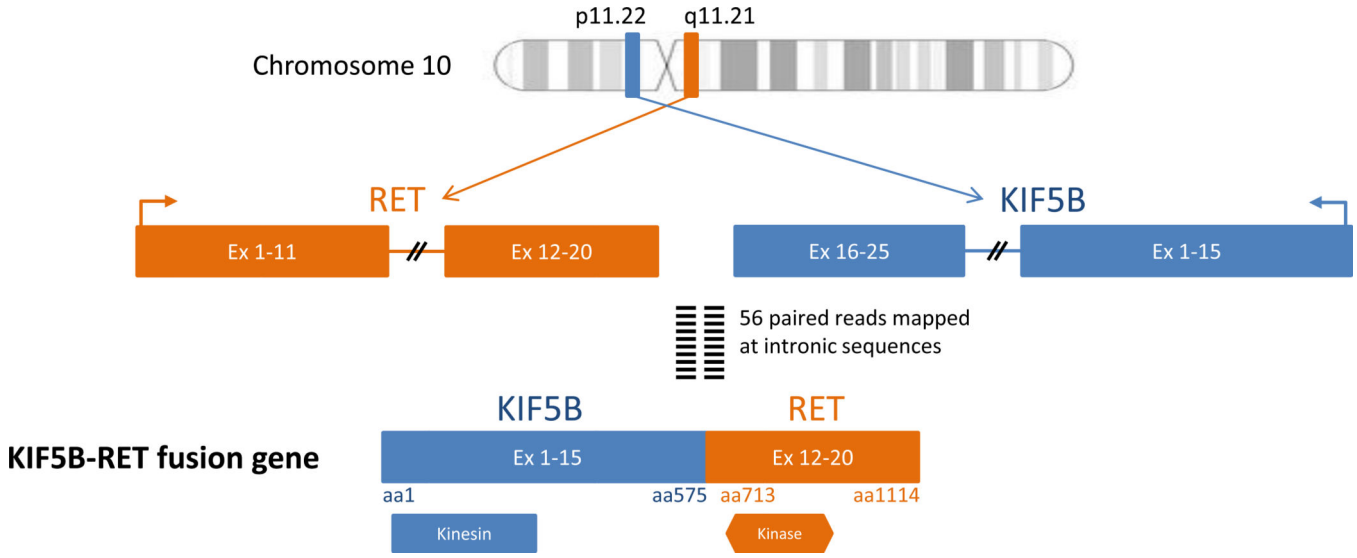

Figure 2.

Schematic representation of KIF5B-RET fusion gene. An inversion rearrangement event within chromosome 10 involves exons 1 to 15 of Kinesin-1 heavy chain protein (KIF5B), containing the kinesin motor domain and exons 12 to 20 of RET proto-oncogene, containing the protein tyrosine kinase domain.

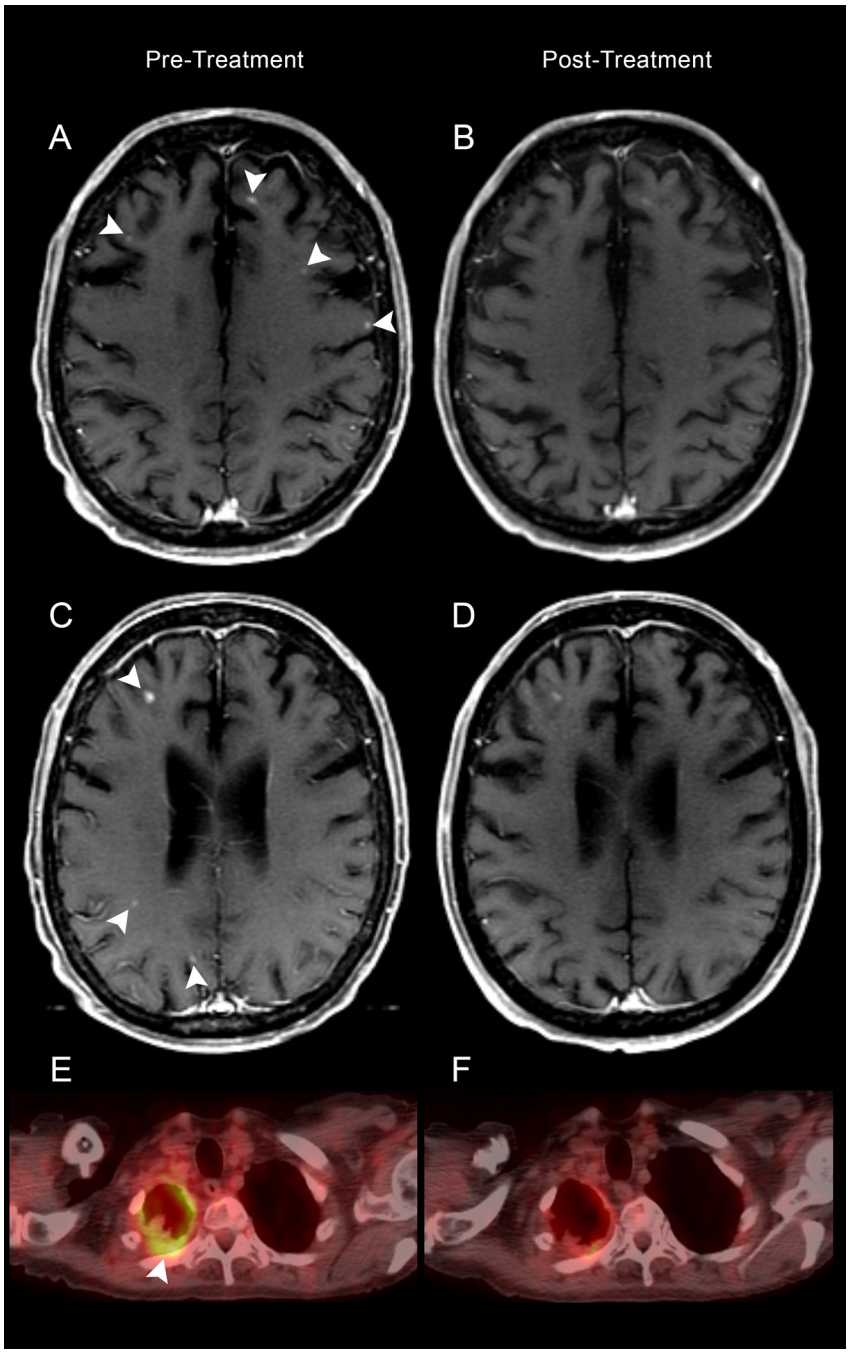

Figure 3.

A–E: Pretreatment MR (A, and C) demonstrates enhancing lesions (arrowheads) on the axial T1-weighted images, decreased in number and size following treatment (B and D). FDG-PET CT reveals pleural hypermetabolism in the right hemithorax (E) with decreased avidity post-therapy (F).

Table 1

Copy number alterations in addition to the KIF5B-RET fusion. Interestingly AKT2 amplification seen is a critical component of the PI3K/mTOR pathway, alterations of which has been associated with both de novo and acquired resistance to targeted therapy. The addition of the rapamycin analog everolimus may have both overcome the AKT amplification to produce a response in addition to its direct effects on the RET gene. In addition there was full homozygous loss of CDKN2A/B.

Gene	Copy Number	Position
MDM2	9	chr12:69153996-69277205
NFKBIA	10	chr14:35871177-35873850
NKX2-1	10	chr14:36947748-37023501
CCNE1	8	chr19:30253918-30360638
AKT2	8	chr19:40701261-40791492
AXL	8	chr19:41725275-41765809
CDKN2A	0	chr9:21853212-21998002
CDKN2B	0	chr9:21998748-22101832