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Patient-driven discovery and post-clinical validation of NTRK3 fusion as an acquired resistance mechanism to selpercatinib in RET fusion-positive lung cancer

V. Subbiah[1,*], **T. Shen**[2,3], **M. Tetzlaff**[4], **A. Weissferdt**[4], **L. A. Byers**[5], **T. Cascone**[5], **A. Behrang**[6], **F. Meric-Bernstam**[1], **B. H. M. Mooers**[2,7], **S. M. Rothenberg**[8,9], **K. Ebata**[8], **J. Wu**[2,3,*]

[1]Departments of Investigational Cancer Therapeutics, the University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA

[2]Peggy and Charles Stephenson Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA

[3]Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA

[4]Anatomical Pathology, the University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA

[5]Thoracic-Head and Neck Medical Oncology, the University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA

[6]Division of Radiology, the University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA

[7]Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA

[8]Loxo Oncology at Lilly, Stamford, CT 06901, USA

[9]Current address: Pfizer Boulder Research and Development, Boulder, CO 80301, USA

Selpercatinib is a RET tyrosine kinase inhibitor (TKI) recently approved for treating RET-altered non-small cell lung cancer and thyroid cancers.[1] Secondary RET mutations and *MET* amplification have been identified as mechanisms of resistance to selpercatinib.[2–5] However, other mechanisms of selpercatinib resistance may exist.

A 62-year-old man with high-grade neuroendocrine carcinoma of thoracic origin developed skin, liver, and intracranial metastases (Supplementary Information, Supplementary Figure S1). He received whole brain radiation, and chemotherapy with carboplatin and etoposide (Figure 1A). Post 5 cycles evaluation showed disease progression (Figure 1B, Supplementary Figure S2). Cell-free DNA (cfDNA) analysis (Guardant) revealed *KIF5B*-

[*]Correspondence to: Vivek Subbiah, UT MD Anderson Cancer Center, 1515 Holcombe Blvd., Unit 455, Houston, TX 77030, USA Tel. 713-563-0393; vsubbiah@mdanderson.org; or Kevin Ebata, Loxo Oncology at Lilly, Stamford, CT 06901, USA, Tel. 650-392-0014, kevin@loxooncology.com; or Jie Wu, University of Oklahoma Health Sciences Center, 975 NE 10th Street, BRC413, Oklahoma City, OK 73104, USA, Tel. 405-271-8001 ext. 31092, jie-wu@ouhsc.edu.

RET fusion (variant allele frequency [VAF] 8.4%) and *PTEN F241fs* mutation (VAF 17.8%). He was then enrolled on the selpercatinib LIBRETTO-001 trial (NCT03157128) starting at 120 mg orally twice daily. Within 2 weeks of starting treatment he showed an overall improved performance status and the serum bilirubin was normalized. At Cycle 4, CT imaging indicated a confirmed partial response per RECIST V1.1 (39% tumor reduction, Figure 1B). His dose of selpercatinib was increased to 160 mg orally twice daily, with subsequent deepening of tumor response to 44% tumor reduction. After 10 months of treatment, CT imaging showed increased liver lesions and MRI revealed innumerable new brain metastases (Figure 1B), the patient's clinical performance status worsened and he developed rapidly rising hyperbilirubinemia, and the patient and family elected to hospice care.

cfDNA analysis from blood samples collected serially during treatment revealed a *KIF5B-RET* fusion (16.3% VAF), *PTEN* F241fs*15 mutation (37.2% VAF), and *EGFR* copy number variation (CNV: 2.4) at screening (Figure 1C, Supplementary Table S1). During treatment with selpercatinib, these variant alleles decreased by ~98% in two weeks, and remained low for 4 months. At the time of disease progression, all three co-variants again increased to higher levels. No RET kinase domain mutations were detected in cfDNA at any time point.

NGS analysis (TST-170, Illumina, Inc.) was performed on the pre-treatment tumor biopsy (skin) and a resistant tumor biopsy (liver). A *KHDRBS1-NTRK3* fusion (K8;N14) and the *KIF5B-RET* fusion (K15;R12) were detected in the resistant tumor sample, while only the *KIF5B-RET* was detected in the pre-treatment sample (Figure 1D, E; Supplementary Figure S3). The *KHDRBS1-NTRK3* fusion was not detected in cfDNA at any time point because the Guardant assay was not designed to detect *NTRK3* fusions.

In cell cultures, KHDRBS1-NTRK3 transformed BaF3 cells into interleukin-3 independence, which was inhibited by the Trk kinase inhibitor larotrectinib but not by selpercatinib, indicating that KHDRBS1-NTRK3 is an oncogenic tyrosine kinase (Figure 1F, Supplementary Figure S4). As expected, KIF5B-RET-transformed BaF3 cells were sensitive to selpercatinib but not to larotrectinib. Importantly, BaF3 cells co-expressing KIF5B-RET and KHDRBS1-NTRK3 were resistant to selpercatinib. Co-treatment of these cells with selpercatinib and larotrectinib suppressed active ERK1/2 and AKT, and induced apoptosis of these cells (Figure 1F, Supplementary Figure 4).

Together, these results highlight the importance of real-time incorporation of molecular profiling results into clinical care, and identify a novel, targetable oncogenic fusion as a new resistance mechanism in KIF5B-RET fusion cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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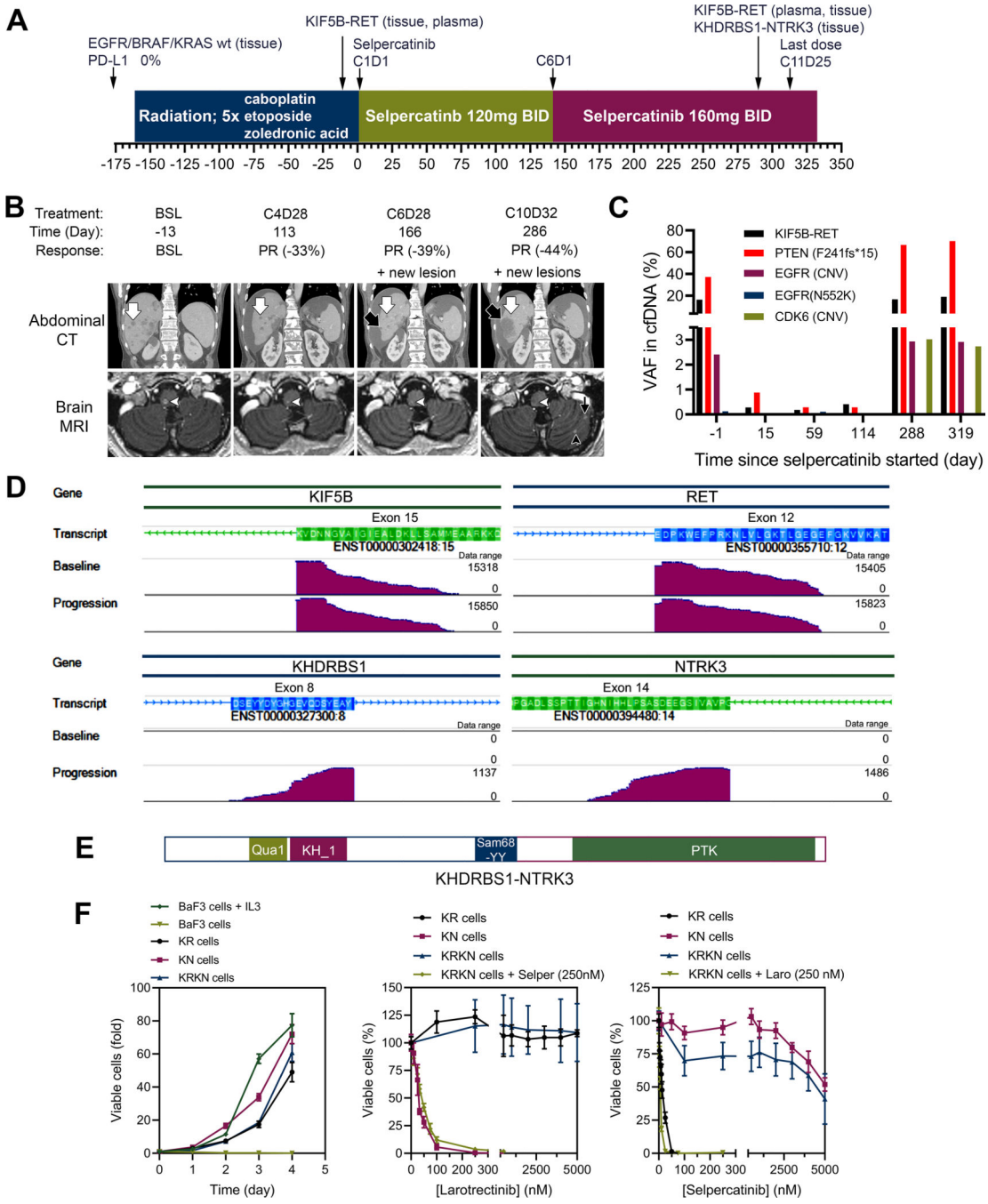

Figure 1.

Treatment history and responses to selpercatinib. (A) Treatment history of a patient with metastatic high-grade neuroendocrine carcinoma of thoracic origin. (B) Serial coronal reformations from a contrast-enhanced abdominal CT (top row) and axial images from contrast-enhanced brain MRI (bottom row) in a 62-year-old man. There are innumerable hepatic and brain lesions, which decrease in size on therapy (examples indicated by white arrow in the liver and white arrowhead in the medulla oblongata). New lesions appear in the liver during cycle 6 (black arrow) and in the brain in cycle 7 (black arrowhead and black thin

arrow) and continue to grow on follow-up scans. (C) Levels of variant alleles detected in cfDNA during the course of selpercatinib treatment. (D) Genome browser images illustrating alignments of fusion supporting sequencing reads of cDNA from baseline and progression tissue samples. (E) Schematic presentation of the KHDRBS1-NTRK3 chimeric protein. (F) The parental BaF3 cells required interleukin-3 (IL3) whereas BaF3/KIF5B-RET (KR), BaF3/KDHRBS1-NTRK3 (KN), and BaF3/KIF5B-RET/KDHRBS1-NTRK3 (KRKN) were IL3-independent (left). Cell viability assay of KR, KN, and KRKN cells treated with selpercatinib (Selper), larotrectinib (Laro), or combination of the two drugs.