

BRIEF COMMUNICATION

Sensitivity to Entrectinib Associated With a Novel LMNA-NTRK1 Gene Fusion in Metastatic Colorectal Cancer

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Abstract

In metastatic colorectal cancer (CRC), actionable genetic lesions represent potential clinical opportunities. *NTRK1*, 2, and 3 gene rearrangements encode oncogenic fusions of the tropomyosin-receptor kinase (TRK) family of receptor tyrosine kinases in different tumor types. The *TPM3-NTRK1* rearrangement is a recurring event in CRC that renders tumors sensitive to TRKA kinase inhibitors in preclinical models. We identified abnormal expression of the TRKA protein in tumor and liver metastases of a CRC patient refractory to standard therapy. Molecular characterization unveiled a novel *LMNA-NTRK1* rearrangement within chromosome 1 with oncogenic potential, and the patient was treated with the pan-TRK inhibitor entrectinib, achieving partial response with decrease in hepatic target lesions from 6.8 and 8.2 cm in longest diameter to 4.7 and 4.3 cm, respectively. To our knowledge, this is the first clinical evidence of efficacy for therapeutic inhibition of TRKA in a solid tumor, illuminating a genomic-driven strategy to identify CRCs reliant on this oncogene to be clinically targeted with entrectinib.

In a pan-cancer analysis of the transcriptomes of nearly 7000 tumors from The Cancer Genome Atlas, it has been reported that *NTRK1* (neurotrophic tyrosine kinase, receptor, type 1), *NTRK2*, and *NTRK3* fusions occur across different tumors including CRC, indicating that such events represent a mechanism of oncogenic activation for this family of receptor tyrosine kinases (1). We recently showed that the *TPM3-NTRK1* rearrangement is a low-frequency (about 1%) recurring event in CRC,

encoding a *TPM3-TRKA*-activated chimeric protein that renders tumors sensitive to tropomyosin receptor kinase A (TRKA) kinase inhibitors in preclinical models (2). We furthermore described an immunohistochemistry (IHC) approach to screen for tumors with rearranged TRKA, based on expression of its kinase domain. These studies provided the rationale for clinical investigation in CRC of the antitumoral activity of entrectinib (RXDX-101, NMS-E628), a novel, highly potent, and selective TRK,

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ROS1 proto-oncogene receptor tyrosine kinase (ROS), and anaplastic lymphoma kinase (ALK) inhibitor (2–6).

A woman age 75 years with metastatic CRC progressing without having had any objective response to previous therapies was referred to Niguarda Cancer Center for experimental targeted therapies. The patient presented with Eastern Cooperative Oncology Group performance status 0, an intact primary colon tumor, peritoneal carcinomatosis and liver metastases, in hepatic segments 6 and 5 of 6.8 and 8.2 cm in longest diameter, respectively, and right adrenal gland deposit of 2.2cm. The primary tumor biopsied in August 2013 was colon adenocarcinoma (Figure 1A). The patient underwent molecular screening, performed ad hoc on a liver biopsy (March 2014), for which the patient provided informed consent and which displayed wild-type RAS and BRAF. We then tested for aberrancies of ALK, ROS1, and NTRK1 genes within the phase I, first-in-human study of entrectinib (EudraCT Number: 2012-000148-88) and found by IHC that expression of TRKA protein was high in both the primary tumor as well as in liver metastasis (Figure 1B; Supplementary Figure 1, available online). However, the fluorescence-in situ hybridization (FISH) pattern (Figure 1C) was unexpectedly different from that observed for the TPM3 (exon 1–7)-NTRK1 (exon 9–16) rearrangement previously reported to occur in CRC as the result of an intrachromosomal inversion within chromosome 1 (Supplementary Figure 2, available online) (2–7). The FISH pattern in this case rather suggested a deletion within Chromosome 1 involving the NTRK1 gene. We therefore investigated this hypothesis, taking advantage of a patient-derived tumor xenograft generated from the liver biopsy in compliance with European and Italian Guidelines for Laboratory Animal Welfare, which mirrored histological, immunohistochemical, and FISH characteristics of the original tumor (Figure 1, D–F). Using a 5'RACE PCR approach, we unveiled a novel LMNA-NTRK1 gene rearrangement, involving loss of the 5' end of the NTRK1 gene, confirmed at genomic level by direct sequencing. Different proteins are produced by alternative splicing of the LMNA gene within exon 10–11, including Lamin A, Lamin C, and Progerin (8). Characterization by

Sanger sequencing of the LMNA-NTRK1 rearrangement identified two distinct splice variant mRNAs, encoding exons 1–10 or 1–11 of the LMNA gene fused to exons 10–16 of the NTRK1 gene (Figure 2; Supplementary Figure 3, available online). Western blot analysis of tumor protein lysate with an antibody recognizing the C-terminus of TRKA revealed the presence of a doublet protein band at molecular weights consistent with those predicted for the two splice variant chimeric proteins. As expected, the two bands were also recognized by anti-Lamin A/C antibody (Figure 2C). The degree of phosphorylation of the highly expressed fusion proteins indicates constitutive activation of TRKA kinase. The downstream transducers PLC γ 1, AKT, and MAPK were also phosphorylated, similar to what was previously reported for TPM3-TRKA in the KM12 CRC cell line (Figure 2D) (2).

Based on these findings, the patient was treated with entrectinib, starting in March 2014, with 1600mg/m² orally once daily for four consecutive days a week (ie, 4 days on/3 days off), for three consecutive weeks every 28 days. At a first response assessment, a CT scan in April 2014 (4 weeks after beginning entrectinib treatment) showed partial response, with shrinkage in sum of target lesions of 30%, which was confirmed by a CT scan performed four weeks later and maintained until July 2014. In particular, lesions in hepatic segments 6 and 5 decreased from 6.8 and 8.2cm in longest diameter to 4.7 and 4.3cm, respectively (Supplementary Figure 4, available online); right adrenal gland metastases and peritoneal nodules also decreased in size (Supplementary Figure 5, available online).

Oncogenic fusions produced by translocations or other chromosomal rearrangements have only infrequently been found in CRC (1). Here, we describe a novel LMNA-NTRK1 gene fusion in this disease. The LMNA gene has recently been identified (together with TP53) as involved in genomic alterations with NTRK1 occurring at relatively high frequency in Spitz nevi (10.7%), atypical Spitz tumors (25%), and Spitzoid melanoma (21.2%) (9). The LMNA-NTRK1 rearrangement reported here differs from that occurring in Spitzoid tumors and encompasses most of the LMNA gene fused to exon 10–16 of the NTRK1 gene, comprising an intact

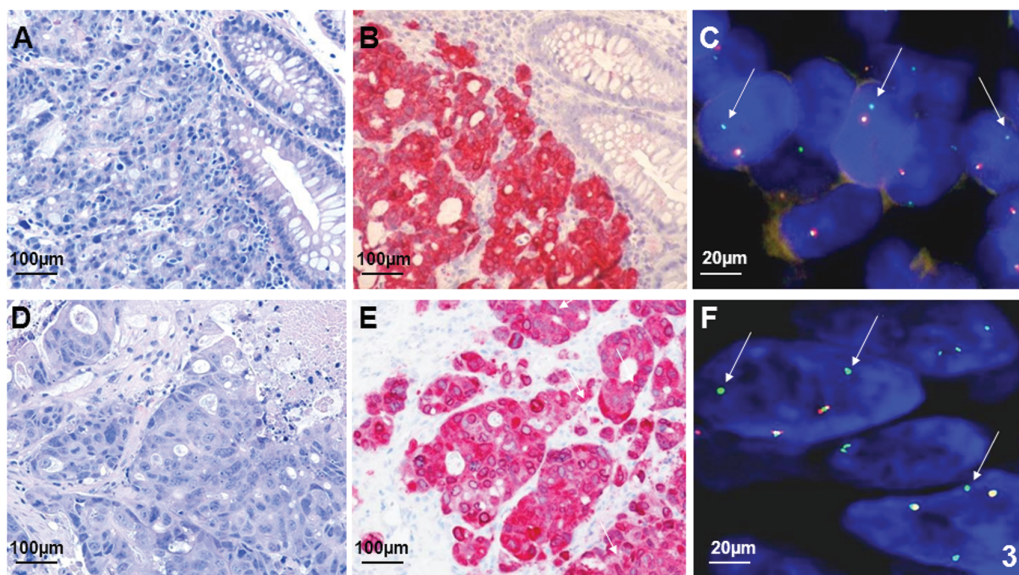


Figure 1. Histologic, immunohistochemical, and fluorescence in situ hybridization analyses of primary tumor and patient-derived xenograft from liver metastasis of the case presented. Hematoxylin and eosin, immunohistochemical and fluorescent in situ hybridization (FISH) images of primary tumor (A–C) and patient-derived xenograft from liver metastasis (D–F). In the immunohistochemical assays, NTRK1 antibody (TrkA Clone ID EP1058Y rabbit monoclonal antibody, EPITOMICS dil. 1:200) shows a strong cytoplasmic reactivity only in the neoplastic component (B, E). In the FISH analyses (C, F) the break-apart probe, which covers the NTRK1 locus (Supplementary Figure 2, available online), shows presence of green signals (white arrows) only in absence of the red ones, suggesting a deletion of the NTRK1 gene. Magnifications of images are 200X for (A, B, D, E) and 630X for (C and F).

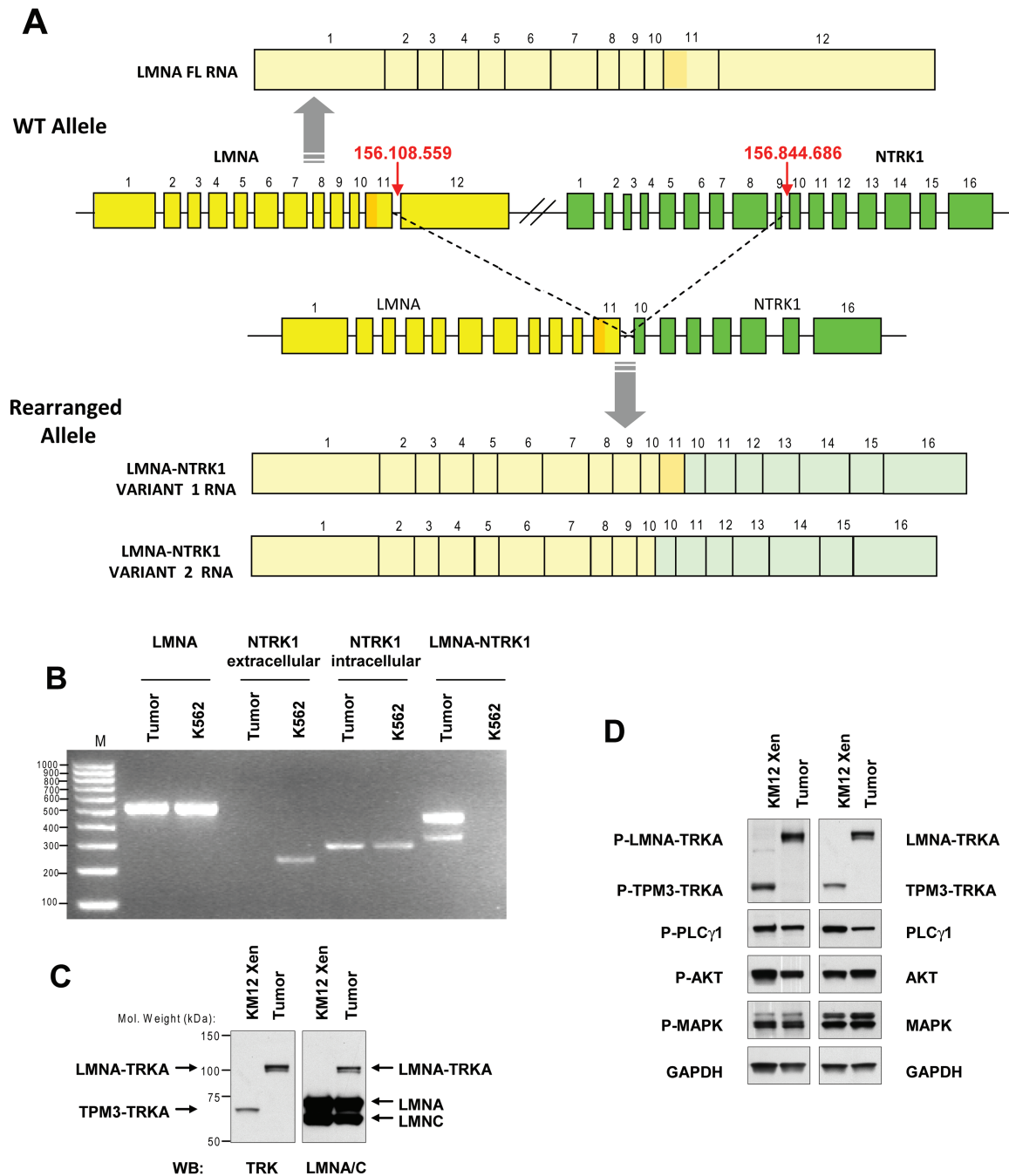


Figure 2. Identification of the new LMNA-NTRK1-activating rearrangement. **A)** Schematic representation of the LMNA-NTRK1 genomic DNA rearrangement and of the resulting transcripts. Introns are reported as lines, exons as colored boxes: LMNA in yellow, NTRK1 in green. The upper section shows in red the breakpoints leading to a deletion within chromosome 1 mapped to the genomic DNA (hg19 version), as identified by PCR/Sanger sequencing. Shown in the lower panel are the rearranged allele and the two identified alternative transcript variants, with or without the LMNA exon 11. **B)** Characterization of LMNA-NTRK1 transcripts by reverse transcription polymerase chain reaction (RT-PCR). The K562 control cell line, which expresses full-length NTRK1, shows expression of both NTRK1 extracellular and intracellular regions, while the tumor sample selectively expresses the intracellular region. PCR analysis of the region spanning the rearrangement shows the presence of two bands that, upon Sanger sequencing, correspond to two alternative splicing variants, with exons 1–10 or 1–11 of LMNA fused in frame to NTRK1 exon 10–16. LMNA expression was used as PCR control. **C and D)** Characterization of the tumor protein lysate sample by western blot. Analysis was performed with antibodies against C-terminus of TRKA (rabbit monoclonal, clone C17F1, 1:1000 dilution, cell signaling) or against Lamin A/C (mouse monoclonal, clone 4C11, 1:2000 dilution, cell signaling). KM12 xenograft lysate was used as a reference (**C**). Phosphorylation of LMNA-TRKA and its downstream targets PLC γ 1, AKT, and MAPK was analyzed using the following antibodies: phospho-TRKA-Tyr490 (rabbit polyclonal, 1:500 dilution, Calbiochem), Phospho-PLC γ 1-Tyr783, PLC γ 1, AKT, Phospho-MAPK-Thr202/Tyr204, MAPK (rabbit polyclonals, 1:1000 dilution, cell signaling), Phospho AKT Ser473 (rabbit monoclonal, clone D9E, 1:1000 dilution, cell signaling). GAPDH (rabbit polyclonal 1:2500 dilution, Santacruz) is reported for normalization purposes (**D**). TRK = tropomyosin-receptor kinase; WT = wild-type.

kinase domain. Alternative splicing at exon 10–11 of the LMNA gene, described in the literature as producing the splicing variants encoding Progerin and Lamin C, generates two different chimeric

transcripts with NTRK1. LaminA/C are structural components of the nuclear lamina, a protein network underlying the inner nuclear membrane, which are highly expressed in colon tissue (8).

The limitation of the present study resides in reporting a single case. However, the rarity of occurrence of fusion genes in CRC precludes clinical studies in large series. The latter will be possible only if pharmaceutical interest and rationale will be triggered by knowledge of successful uncommon cases such as the present one. Furthermore, if applied routinely a screening based on NTRK assessment by immunohistochemistry followed by targeted NGS based on anchored multiplex polymerase chain reaction (10) will enhance identification of patients who can benefit from entrectinib treatment in CRC and other histologies.

Entrectinib is a selective pan-TRK, ROS1, and ALK kinase inhibitor with strong preclinical activity in multiple cancer models where these targets are constitutively activated (2–4) and which is currently being developed in phase I clinical studies for tumors with TRK, ROS, or ALK gene aberrations (6). While clinical responses to ALK and ROS inhibitors have already been observed in lung tumor patients treated with crizotinib, ceritinib, alectinib, and other drugs in development, including entrectinib, clinical responses to TRK inhibition have never, to our knowledge, previously been documented. The landscape of therapeutically actionable molecular alterations in CRC appears to be increasingly populated by myriad gene abnormalities, among which NTRK gene rearrangements encoding novel oncogenic fusions (2,11,12) are promising targets to be captured by rapidly evolving technologies (10).

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