Tropomyosin receptor kinase (TRK) biology and the role of NTRK gene fusions in cancer

A. Amatu1†, A. Sartore-Bianchi1,2†, K. Bencardino1, E. G. Pizzutilo1,2, F. Tosi1,2 & S. Siena1,2*

1Department of Hematology and Oncology, Niguarda Cancer Center, Grande Ospedale Metropolitano Niguarda, Milan; 2Department of Oncology and Hematology, Università degli Studi di Milano, Milan, Italy

*Correspondence to: Prof. Salvatore Siena, Department of Hematology and Oncology, Niguarda Cancer Center, Grande Ospedale Metropolitano Niguarda, Piazza Ospedale Maggiore, 3, 20162 Milan, Italy. Tel: +39-02-6444-2291; E-mail: salvatore.siena@unimi.it

†Both authors contributed equally to this work.

The tropomyosin receptor kinase (TRK) family of receptor tyrosine kinases are encoded by NTRK genes and have a role in the development and normal functioning of the nervous system. Since the discovery of an oncogenic NTRK gene fusion in colorectal cancer in 1986, over 80 different fusion partner genes have been identified in a wide array of adult and paediatric tumours, providing actionable targets for targeted therapy. This review describes the normal function and physiology of TRK receptors and the biology behind NTRK gene fusions and how they act as oncogenic drivers in cancer. Finally, an overview of the incidence and prevalence of NTRK gene fusions in various types of cancers is discussed.

Key words: TRK, tropomyosin receptor kinase, NTRK gene fusions, TRK fusion cancer

Introduction

The identification of gene fusions in a variety of cancers has provided actionable targets that have expanded therapeutic options and facilitated precision medicine. These gene aberrations result in the expression of fusion proteins with constitutive activity that become oncogenic drivers [1]. The tropomyosin receptor kinase (TRK) family of receptor tyrosine kinases are of interest as the NTRK genes that encode them are involved in gene fusions identified in a wide range of adult and paediatric tumours.

In this review, we discuss the normal function and physiology of TRK receptors, the biology behind NTRK gene fusions, the mechanisms by which NTRK gene fusions become oncogenic drivers in cancer, and the incidence and prevalence of NTRK gene fusions in a variety of cancers.

Normal function and physiology of NTRK genes and TRK receptors

Structure. TRKA, TRKB and TRKC are transmembrane proteins that comprise the TRK receptor family. TRKA is encoded by the NTRK1 gene located on chromosome 1q21-q22 [2]. TRKB is encoded by the NTRK2 gene located on chromosome 9q22.1 [3]. TRKC is encoded by the NTRK3 gene located on chromosome 15q25 [4]. Each of the TRK receptors consists of an extracellular domain, a transmembrane region and an intracellular region containing the tyrosine kinase domain. The extracellular domain contains a cysteine-rich cluster (C1) followed by three leucine-rich 24-residue repeats (LRR1–3), another cysteine-rich cluster (C2) and two immunoglobulin-like domains (Ig1 and Ig2; Figure 1) [5–7]. The LRR1–3 motifs are specific to TRK proteins and are not found in other receptor tyrosine kinases [6]. The intracellular region contains five key tyrosine residues (Figure 1): three within the activation loop of the kinase domain, which are necessary for full kinase activity, and two on either side of the tyrosine kinase domain, which serve as phosphorylation-dependent docking sites for cytoplasmic adaptors and enzymes [8].

TRK receptors and associated ligands. The TRK receptors are activated by a family of four proteins called neurotrophins. Neurotrophins were initially identified as survival molecules for sensory and sympathetic neurons [9], but are now understood to play many roles in the development and function of the nervous system [10]. Each of the four neurotrophins have specificity for a particular TRK and bind to it with high affinity (Figure 1). Nerve growth factor (NGF) binds to TRKA [11, 12], both brain-derived...
neurotrophin 4 (BDNF) and neurotrophin 4 (NT-4) bind to TRKB [13–15] and neurotrophin 3 (NT-3) binds to TRKC [16]. NT-3 can bind to all three TRK receptors but has highest affinity for TRKC and is its sole ligand [14, 15, 17, 18]. Alternative splicing of TRK proteins can alter the interaction between a TRK receptor and its specific neurotrophin (Figure 2) [10, 19]. For example, short amino acid sequence insertions observed in the juxtamembrane region of the extracellular domains of TRKA and TRKB enhance their binding with non-cognate ligands [20, 21]. Isoforms of TRKA and TRKB that lack this insertion are activated strongly only by NGF and BDNF, respectively. In contrast, with this insertion, the TRKA splice variant is activated by NT-3 in addition to NGF [20] and the TRKB splice variant is readily activated by NT-3 and NT-4 in addition to BDNF [21]. Alternative splicing of exons encoding parts of the intracellular domains of TRK receptors may also affect downstream signalling initiated by neurotrophin binding to the receptor. Such alternatively spliced TRKB and TRKC isoforms have been observed to contain comparatively short cytoplasmic motifs missing the tyrosine kinase domain, leading to a lack of receptor response to neurotrophins [22]. For example, alternative splicing of the NTRK3 gene may lead to amino acid insertion into the TRKC tyrosine kinase domain, which in turn results in modified kinase substrate specificity and impaired ability to promote neuronal cell differentiation [23].

Role in development and physiology. TRK receptors are predominantly expressed in neuronal tissue and play an essential role during embryonic development as well as in the normal function of the nervous system [7, 26]. The activation of TRK receptors by neurotrophins has an impact on a variety of neuronal events, such as neuronal cell differentiation and survival, cell proliferation, synaptic formation and plasticity, membrane trafficking, and axon and dendrite formation [7, 19, 27].

TRK receptors and their respective neurotrophins have been implicated in memory formation and retention, nociception and proprioception [31, 32], as well as having roles in non-neuronal tissues including the vasculature, ovaries and immune system [33–36]. Loss-of-function mutations in NTRK genes can result in...
several diseases, indicating the role of TRK receptors in normal regulation and function. TRKA receptors are involved in pain sensation; loss-of-function mutations in TRKA are observed in class IV hereditary sensory and autonomic neuronal disorders (such as congenital insensitivity to pain with anhidrosis), which result in impaired ability to sense differences in temperature or feel pain [37, 38]. Loss-of-function mutations in TRKB result in energy imbalances, loss of appetite control and subsequent obesity, in addition to defects in learning, memory and nociception [39–41].

Discovery of aberrant gene fusions and ligand-independent oncogenic proteins

Discovery of NTRK gene fusions in cancer. Somatic fusions involving the NTRK genes were first observed in a patient with colorectal cancer (CRC) in 1986, when Martin-Zanca et al. identified a chimeric fusion oncogene resulting from an intrachromosomal rearrangement at 1q22-23 [42]. This oncogene involved the tropomyosin 3 gene (TPM3) and a locus that was subsequently cloned and found to encode a high-affinity NGF receptor (NTRK1) [12]. Following the discovery of this TPM3-NTRK1 gene fusion, the identification of other NTRK gene fusions in CRC [43–45] triggered the interest of clinicians in the possible existence of oncogenic gene fusions in other types of cancers; to date, over 80 different fusion partner genes have been identified in a wide array of tumours (Figure 4).

Oncogenic mechanism of NTRK gene fusions. In NTRK gene fusion events, the 3’ region of the NTRK gene is joined with the 5’ end of a fusion partner gene, either by intrachromosomal or interchromosomal rearrangement. The resulting fusion gene encodes a protein containing the N-terminus of the fusion partner joined to the C-terminus of the TRK protein, including the catalytic tyrosine kinase domain [27]. The majority of characterised NTRK gene fusions contain a 5’ partner gene sequence encoding one or more dimerisation domains. These domains mediate the corresponding constitutive tyrosine kinase activity that occurs, thus conferring ligand-independent oncogenic potential through uninterrupted downstream signalling messages, promoting cell proliferation and survival [27].

Incidence and prevalence of NTRK gene fusions

Incidence and prevalence data for NTRK gene fusions have only recently become clearer following the increasing availability of next-generation sequencing (NGS) and comprehensive molecular testing methods. NTRK gene fusions have been identified in two main categories of tumours with vastly differing rates of occurrence; certain rare cancers present with a high frequency (>80%) of NTRK gene fusions, while some more common cancers present with a lower frequency of NTRK gene fusions (<25%) [24, 27, 46]. NTRK gene fusions have been estimated to occur in up to 1% of all solid tumours [27, 46, 47]. Gene fusion events appear to arise more commonly in the NTRK1 and NTRK3 genes, with the possible exception of brain tumours [27, 46–48]. Immunohistochemistry (IHC) screening in 1043 various solid tumours showed TRKA expression in 1.6% of samples, including CRC, lung cancer, biliary tract carcinoma and thyroid cancer. Of note, only 5.9% of these showed NTRK1 gene rearrangements, while 88.2% of cases displayed NTRK1 gene copy number gain without amplification [49]. In a retrospective analysis of 33 997 patients, screening with a targeted DNA-based NGS panel (MSK-IMPACT) identified 87 patients (0.26%) with NTRK1–3 gene fusions. The prevalence of NTRK1–3 gene fusions in this group ranged from 0.13% to 17.7% depending on the various tumour types. Screening with pan-TRK IHC in this study showed better sensitivity than DNA-based NGS.

Figure 2. Known splice variants of TRKA, TRKB and TRKC [6]. C1/C2, cysteine-rich clusters; Ig1/Ig2, immunoglobulin-like domains; KD, kinase domain; LRR1–3, leucine-rich repeats; TM, transmembrane; TRK, tropomyosin receptor kinase.
NTRK gene fusions are pathognomonic in certain rare paediatric and adult cancers. Infantile fibrosarcoma (IFS), a malignant tumour of fibroblasts, represents <1% of all paediatric cancers but is the most commonly occurring non-rhabdomyosarcoma soft tissue sarcoma in children under 1 year of age [47]. IFS is virtually identical histologically to the cellular variant of congenital mesoblastic nephroma (CMN), an infantile spindle cell tumour of the kidney that occurs in the same age group and represents ~5% of all childhood renal neoplasms. In 1998, Knezevic et al. discovered a recurrent ETV6-NTRK3 gene fusion in IFS, which was found to occur in ~70% of cases of IFS [51]. The same year, two other groups identified the same ETV6-NTRK3 gene fusion in the cellular variant of CMN, establishing a genetic link between IFS and cellular CMN [52, 53]. Thereafter, identification of the ETV6-NTRK3 translocation has become a useful diagnostic marker for IFS/CMN, and the presence of this gene fusion is considered pathognomonic for these two rare cancers. Several additional novel translocations involving NTRK genes have subsequently been described in IFS/CMN [54, 55] (Figure 4); consequently, genomic testing using break-apart fluorescence in situ hybridisation specific for ETV6 may be insufficient both as a diagnostic and predictive marker [56].

Secretory breast carcinoma (SBC) is one of the rarest types of breast carcinomas, accounting for ~0.15% of all breast cancers [57]. It is characterised by intracellular and extracellular eosinophilic secretions and usually presents as a triple-negative breast carcinoma with an immunohistochemical profile akin to basallike breast carcinoma. Tognoni et al. first reported an ETV6-NTRK3 gene fusion in 12 out of the 13 cases of SBC by identifying the corresponding chromosomal translocation t(12; 15)(p13; q25) [58].

Mammary analogue secretory carcinoma (MASC) is a rare neoplasm of minor and major salivary glands morphologically and immunohistochemically similar to SBC. Since it was first described in 2010 by Skálová et al. [59], fewer than 300 cases have been reported in the literature [60]. Skálová et al. found that of 14 cases of MASC, all but one was characterised by the ETV6-NTRK3 gene fusion [59]. While ETV6-NTRK3 is the most common gene fusion seen in MASC, other rearrangements involving ETV6 and NTRK1 or NTRK2 have been identified [53]. On the other hand, no partner genes other than ETV6 have been described in cases of MASC harbouring NTRK3 rearrangement (Figure 4).
**Figure 4.** NTRK gene fusions in cancers. *Sinonasal low-grade non-intestinal-type adenocarcinoma, parotid gland acinic cell carcinoma, anaplastic thyroid carcinoma, Erdheim–Chester disease, interdigitating dendritic cell sarcoma. **One large-cell neuroendocrine carcinoma of the lung with COP1-NTRK1, one small-cell lung cancer with ETV6-NTRK3. CMN, congenital mesoblastic nephroma; GIST, gastrointestinal stromal tumour; ICC, intrahepatic cholangiocarcinoma; IFS, infantile fibrosarcoma; MASC, mammary analogue secretory carcinoma; NET, neuroendocrine tumour; NSCLC, non-small-cell lung cancer.
NTRK gene fusions in common cancers. Thyroid cancer: Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer, accounting for 80% of all thyroid cancer cases [61]. Since the identification of NTRK1 as an oncogenic driver in PTC by Bongarzone et al. in 1989 [62], the reported frequency of NTRK1 rearrangement in PTC has been shown to range from <5% to 25% [63–68]. More recently, novel NTRK3 fusion genes have been discovered in PTC, with ETV6-NTRK3 being the most common rearrangement found after any RET-PTC isoform in The Cancer Genome Atlas Project [61]. While the prevalence of ETV6-NTRK3 in PTC in adults is very low (1%), it is the second most common rearrangement seen in radiation-associated PTC [69, 70].

Colorectal and appendiceal cancer: Following the identification of TPM3-NTRK1 as an oncogenic driver in CRC in 1986 [42], the third most common form of cancer, nothing further was reported about this gene fusion until almost 30 years later when Ardini et al. characterised the TPM3-NTRK1 rearrangement at the genomic level for the first time, finding that the observed breakpoint within exon 8 of NTRK1 in CRC differed from those previously identified for the TPM3-NTRK1 gene fusion in PTC. This group also developed and validated an IHC method for the identification of TRKA-positive clinical specimens, offering a readily applicable approach to screening CRC for TRKA overexpression and thus identifying those cases that could potentially benefit from targeted therapy [43]. Further cases of CRC harbouring either NTRK1 or NTRK3 gene fusions involving different partner genes have subsequently been reported and, in some cases, demonstrated pharmacologically actionable (Figure 4) [44, 45, 71–73]. A recent molecular profiling study used a plasma-based cell-free circulating tumour DNA NGS assay to detect gene fusions in 4290 patients with CRC. Using different gene panels, including one testing for NTRK1 (but not NTRK2 or NTRK3) gene fusions, only three (0.07%) cases were detected [74]. These data are consistent with the prevalence previously found using a tissue-based NGS assay [75]. Notably, gene fusions seem to be associated with high mutation burden [74], and microsatellite instability (MSI) is frequently found in CRCs harbouring NTRK gene fusions [44, 71, 76]. Hypothetically, the increased mutational frequency in MSI-high CRCs could explain the higher incidence of NTRK gene rearrangements as well as NTRK mutations [77]. To date, only NTRK2 fusions have been identified in cases of appendiceal adenocarcinoma [73, 78].

Lung cancer: Lung cancer is the leading cause of cancer-related mortality in the world. Non-small-cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for 85% of all lung cancer cases [79]. NTRK1 gene rearrangements in NSCLC were first described in 2013 among a subset of patients with NSCLC with adenocarcinoma histology and no detectable EGFR, KRAS, ALK or ROS1 alterations (3/91; 3.3%) [80]. In a larger and unselected cohort of 1378 patients with NSCLC, NTRK1 gene fusions were detected in two patients (0.1%) [81]. NTRK2 and NTRK3 gene fusions in NSCLC have also been described [48, 82]. Overall, NTRK gene fusions occur at a frequency of ~0.1%–1.0% [27, 80, 81] (Figure 4).

Sarcoma: NTRK gene fusions are relatively rare in soft tissue sarcoma. Testing on 1272 soft tissue sarcoma samples identified eight cases (<1%) with NTRK1 or NTRK3 gene fusions, with one-half of these found in patients under the age of 5 years [83]. Recurrent NTRK1 gene fusions have been noted in soft tissue sarcomas characterised by a prominent myoepithelial/haemangioendothelioma growth pattern [84]. Several studies involving the genetic sequencing of tumour samples have led to the characterisation of novel subtypes of sarcoma not previously described. Undifferentiated uterine sarcoma is a diagnosis of exclusion after more common uterine mesenchymal tumours, such as leiomyosarcoma, have been ruled out. From a database of gynaecological cancer patients, Chiang et al. prospectively identified four NTRK gene fusion-positive undifferentiated uterine sarcomas with spindle cell morphology that were morphologically and immunophenotypically unique from leiomyosarcoma and other undifferentiated uterine sarcoma. This discovery suggested a novel uterine sarcoma subtype defined by the presence of recurrent NTRK gene fusions [85]. Similarly, Agaram et al. described a novel and distinct subset of NTRK1 gene fusion-positive soft tissue tumours occurring in children and young adults resembling lipofibromatosis (LPF) but displaying cytologic atypia and a neural immunophenotype. These tumours have been provisionally named LPF-like neural tumours and are defined by NTRK1 oncogenic activation [86]. ETV6-NTRK3 gene fusions have also been identified in inflammatory myofibroblastic tumours in adolescent and adult patients [87], especially in ALK-negative tumours [88, 89].

Central nervous system cancers: NTRK gene fusions have been identified in both paediatric and adult primary central nervous system (CNS) tumours, including glioblastoma multiforme (GBM), paediatric gliomas and astrocytomas [27]. Frattini et al. analysed 185 samples of GBM and discovered 2 NTRK1 gene fusions (1%) with two different 5’ fusion partners (NFASC-NTRK1 and BCAN-NTRK1) [90]. Several additional NTRK translocations have subsequently been described in GBM (Figure 4). In a series of 127 paediatric high-grade gliomas (HGGs), Wu et al. reported recurrent fusions involving NTRK genes in 4% of diffuse intrinsic pontine gliomas and 10% of non-brainstem HGGs (NBS-HGGs). Notably, 40% (4/10) of NBS-HGGs in children aged <3 years harbour an NTRK1 gene fusion [91]. Different fusions involving NTRK genes have also been reported in low-grade gliomas (Figure 2). Low-grade neuroepithelial tumours (LGNTs) are a diverse group of CNS tumours presenting in children and young adults; pilocytic astrocytomas are the most common LGNT seen in children. Jones et al. used whole-genome sequencing to analyse 96 pilocytic astrocytomas and identified two novel NTRK2 gene fusions (QKI-NTRK2 and NACC2-NTRK2) in three samples [92]. Qaddoumi et al. also utilised whole-genome sequencing to analyse 91 less common LGNTs and identified two tumours harbouring NTRK2 translocations, including a novel SLMAP-NTRK2 gene fusion found in a case of parietal ganglioglioma [93]. NTRK rearrangements have also been reported in diffuse leptomeningeal glioneuronal tumours [94]; rare CNS neoplasms that were included in the 2016 update of the World Health Organization classification [95]. In addition, cancers that can harbour NTRK gene fusions,
such as lung cancers and melanomas, have a proclivity for CNS metastases [27, 96].

**Spitz tumours/melanoma:** Various translocations involving NTRK1 or NTRK3 have been reported in spitzoid melanocytic neoplasms as well as in compound Spitz nevi [97–99]. More recently, an NGS analysis was carried out by Lezcano et al. in order to assess the frequency of NTRK gene rearrangements in non-spitzoid metastatic melanomas. Among 751 cases, they identified three cutaneous primary melanomas (3/395; 0.8%) and one mucosal/paramucosal melanoma (1/113; 0.9%) harbouring NTRK1 or NTRK2 gene fusions [100].

**Other tumour types:** TRK fusions have also been reported in intrahepatic cholangiocarcinomas [101], breast cancer [102], quadruple wild-type (ETV6-NTRK3) gastrointestinal stromal tumours [103, 104], gallbladder adenocarcinomas [73], pancreatic carcinomas [105], sinus–nasal low-grade non-intestinal-type adenocarcinomas [106] and neuroendocrine tumours of the small bowel [107]. In addition to being present in solid tumours, NTRK gene fusions are also detected in acute lymphoblastic leukaemia (ALL) [108] and acute myeloid leukaemia [109] at a frequency of <5% [6].

**Preclinical and clinical evidence that NTRK gene fusions are oncogenic drivers**

Preclinical studies with inhibitors of TRK proteins have further substantiated the role of NTRK gene fusions as oncogenic drivers. Mouse models of genetically engineered NTRK gene fusion-positive cancers have been shown to develop highly aggressive tumours. Two such studies involved a conditional knock-in model of carrying the Etv6-NTRK3 gene fusion [109] and a chromosomal engineered glioma model harbouring the Bcan-Ntrk1 gene fusion [110]. In both models, the tumours were effectively controlled using TRK inhibitors, indicating that the TRK fusion protein was implicated in the proliferation and survival of tumour cells. In a separate in vitro study, analysis of CRC cell lines revealed NTRK1 overexpression that was associated with gene translocation. When this gene was suppressed through the use of short interfering RNA or TRKA inhibition, the ensuing reduction in protein expression or activity significantly impaired cell growth and increased apoptosis, suggesting functional dependency [111]. Furthermore, studies in mice demonstrated that conditional expression of an Etv6-NTRK3 gene fusion was sufficient to initiate mammary tumourigenesis [112]. Importantly, NTRK gene fusions appear to be mutually exclusive to other gene alterations, suggesting that they may act as the sole oncogenic drivers in the tumours that harbour them [48, 82, 113].

Additional preclinical and clinical studies of tyrosine kinase inhibitors have provided further evidence of NTRK gene fusions as oncogenic drivers. Entrectinib (RDX-101, NMS-P626), a multikinase inhibitor, was shown to suppress TPM3-TRKA protein phosphorylation in mice with CRC harbouring a TPM3-NTRK1 gene fusion [43], and further showed efficacy in three clinical trials including patients with NTRK gene fusions [114, 115].

Larotrectinib is a highly selective TRK inhibitor recently approved by the US Food and Drug Administration* for the treatment of adult and paediatric patients with solid tumours that harbour an NTRK gene fusion. Larotrectinib inhibited fusion protein signalling, in vitro proliferation and in vivo tumour growth in models derived from human cancer cells harbouring NTRK gene fusions [80, 97], as well as demonstrated clinical efficacy and safety in three clinical trials [46, 116, 117]. Resistance to larotrectinib and entrectinib can occur through the development of NTRK gene mutations, which involves amino acid substitutions in the solvent-front, gatekeeper residues of the NTRK genes (NTRK1 p. G667C, NTRK3 p. G696A) and xDFG motif substitutions [114, 118]. Second-generation TRK inhibitors, such as selitrectinib (BAY 2731954, LOXO-195), are under clinical development based on their ability to overcome acquired resistance mediated by these acquired recurrent mutations [114].

Other NTRK alterations, such as mutations, amplifications and mRNA overexpression, were found in ~14% of 13 467 adult and paediatric pan-cancer tumour samples obtained from The Cancer Genome Atlas and the St Jude PeCan database [119]. NTRK mutations occur less frequently than amplifications or mRNA overexpression [119], but may be enriched in MSI-high CRCs [77]. These NTRK mutations are different from the acquired mutations described as a resistance mechanism to TRK inhibitors; as expected, the known acquired NTRK mutations that confer resistance were not observed in any of the 13 467 treatment-naı¨ve tumours [119]. NTRK point mutations themselves are generally not activating oncogenic events [120] and have limited response to larotrectinib, as demonstrated in a phase I clinical trial of larotrectinib [117] where none of the patients with NTRK point mutations had an objective response to larotrectinib; in contrast, objective responses were seen in seven of eight patients with tumours harbouring NTRK gene fusions. The oncogenic role of TRK overexpression and NTRK gene amplification also remains unclear [6]. In the same trial with larotrectinib, one patient with a tumour harbouring an NTRK1 gene amplification had a single 11 mm target lesion shrink by 5 mm (45.5%). The duration of response for this patient was 3.7 months, whereas in the patients with TRK fusion cancer the median duration of response had not been reached at a median follow-up of 26.9 months [117].

**Lessons learned**

NTRK gene fusions can be drivers of cancer progression and, as such, their oncogenic products can be therapeutically targeted. Specific NTRK gene fusions have been identified in various tumours and can be found with high prevalence in certain rare adult and paediatric tumour types, even becoming a defining diagnostic feature, and at low prevalence in most common cancers. Advances in both NTRK gene fusion detection and targeted therapies to inhibit TRK are changing the diagnostic and therapeutic landscape of treatment of these cancers [46, 96].

*Note added in proof: The European Medicines Agency granted marketing authorisation for larotrectinib on 23 September 2019 as monotherapy for the treatment of adult and paediatric patients with solid tumours that harbour an NTRK gene fusion.
Acknowledgements

Medical writing support, including assisting authors with the development of the outline and initial draft, incorporation of comments, and preparation of tables and figures, was provided by Cindy Cheung, MBBS; editorial support, including fact-checking, referencing, figure preparation, formatting, proof-reading and submission, was provided by Annabel Ola, MSc; both of Scion (London, UK), supported by Bayer Healthcare according to Good Publication Practice guidelines (https://annals.org/aim/fullarticle/2424869/good-publication-practice-communicating-company-sponsored-medical-research-gpp3).

Funding

This work is supported in part by the Associazione Italiana Ricerca Cancro (AIRC) 5×1000 Special Program – Molecular Clinical Oncology [grant number 51000]. SS is supported by the AIRC Investigator Grant [grant number 20685], the AIRC 5×1000 Special Program – Metastases [grant number 21091], Community Research and Development Information Service (CORDIS) Horizon 2020 – Molecurly Guided Trials with Specific Treatment Strategies in Patients with Advanced Newly Molecular Defined Subtypes of Colorectal Cancer (MoTriColor) [grant number 655342] and Fondazione Regionale Ricerca Biomedica [grant number IANG-CRC CP2_12/2018]. AS-B is supported by Fondazione Oncologia Niguarda Onlus, grant Terapia Molecolare dei Tumori to AS-B [no grant number applicable]. SS and AS-B are supported by the Studies to Develop Specific Treatment Strategies in Patients with Advanced Newly Molecular Defined Subtypes of Colorectal Cancer in Young Adults [grant number 12018]. This paper was published as part of a supplement financially supported by Bayer AG and Loxo Oncology, Inc., a wholly owned subsidiary of Eli Lilly and Company.

Disclosure

SS is an advisory board member for Amgen, Bayer, BMS, CheckmAb, Celgene, Daichi-Sankyo, Incyte, Merck, Novartis, Roche-Genentech and Seattle Genetics. AA is an advisory board member for Amgen, Bayer and Roche. AS-B is an advisory board member for Amgen, Bayer and Sanofi. All remaining authors have declared no conflicts of interest.

References

1. Schram AM, Chang MT, Jonsson P et al. Fusions in solid tumours: diagnostic strategies, targeted therapy, and acquired resistance. Nat Rev Clin Oncol 2017; 14(12): 735–748.
2. Weier HU, Rhein AP, Shadravan F et al. Rapid physical mapping of the human trk protooncogene (NTRK1) to human chromosome 1q21–q22 by P1 clone selection, fluorescence in situ hybridization (FISH), and computer-assisted microscopy. Genomics 1995; 26(2): 390–393.
3. Nakagawa A, Liu XG, Ikegaki N et al. Cloning and chromosomal localization of the human TRK-B tyrosine kinase receptor gene (NTRK2). Genomics 1995; 25(2): 538–546.
4. Valient A, Danglot G, Bernheim A. Mapping of the tyrosine kinase receptors trkA (NTRK1), trkB (NTRK2) and trkC (NTRK3) to human chromosomes 1q22, 9q22 and 15q25 by fluorescence in situ hybridization. Eur J Hum Genet 1997; 5(2): 102–104.
5. Skaper SD. The biology of neurotrophins, signalling pathways, and functional peptide mimetics of neurotrophins and their receptors. CNS Neurol Discord Drug Targets 2008; 7(1): 46–62.
6. Cocco E, Sclafitti M, Drilon A. NTRK fusion-positive cancers and TRK inhibitor therapy. Nat Rev Clin Oncol 2018; 15(12): 731–747.
7. Reichardt LF. Neurotrophin-regulated signalling pathways. Phil Trans R Soc B 2006; 361(1473): 1545–1564.
8. Cunningham ME, Greene LA. A function-structure model for NGF-activated TRK. EMBO J 1998; 17(24): 7282–7293.
9. Cohen S, Levi-Montalcini R, Hamburger V. A nerve growth-stimulating factor isolated from sarcomas 37 and 180. Proc Natl Acad Sci USA 1954; 40(10): 1014–1018.
10. Barbacid M. The Trk family of neurotrophin receptors. J Neurobiol 1994; 25(11): 1836–1840.
11. Kaplan DR, Hempstead BL, Martin-Zanca D et al. The trk proto-oncogene product: a signal transducing receptor for nerve growth factor. Science 1991; 252(5005): 554–558.
12. Kaplan DR, Martin-Zanca D, Parada LF. Tyrosine phosphorylation and tyrosine kinase activity of the trk proto-oncogene product induced by NGF. Nature 1991; 350(6314): 158–160.
13. Klein R, Nanduri V, Jing SA et al. The trkB tyrosine protein kinase is a receptor for brain-derived neurotrophic factor and neurotrophin-3. Cell 1991; 66(2): 395–403.
14. Soppet D, Escandon E, Maragos J et al. The neurotrophic factors brain-derived neurotrophic factor and neurotrophin-3 are ligands for the trkB tyrosine kinase receptor. Cell 1991; 65(5): 895–903.
15. Squinto SP, Stitt TN, Aldrich TH et al. trkB encodes a functional receptor for brain-derived neurotrophic factor and neurotrophin-3 but not nerve growth factor. Cell 1991; 65(5): 885–893.
16. Lamballe F, Klein R, Barbacid M. trkC, a new member of the trk family of tyrosine kinase kinases, is a receptor for neurotrophin-3. Cell 1991; 66(3): 967–979.
17. Ip NY, Stitt TN, Tapley P et al. Similarities and differences in the way neurotrophins interact with the Trk receptors in neuronal and non-neuronal cells. Neuron 1993; 10(2): 137–149.
18. Cordón-Cardo C, Tapley P, Jing SQ et al. The trk tyrosine protein kinase mediates the mitogenic properties of nerve growth factor and neurotrophin-3. Cell 1991; 66(1): 173–183.
19. Huang EJ, Reichardt LF. Trk receptors: roles in neuronal signal transduction. Annu Rev Biochem 2003; 72: 609–642.
20. Clary DO, Reichardt LF. An alternatively spliced form of the nerve growth factor receptor TrkA confers an enhanced response to neurotrophin 3. Proc Natl Acad Sci USA 1994; 91(23): 11133–11137.
21. Strohmaier C, Carter BD, Urfer R et al. A splice variant of the neurotrophin receptor trkB with increased specificity for brain-derived neurotrophic factor. EMBO J 1996; 15(13): 3332–3337.
22. Eide FF, Vining ER, Eide BL et al. Naturally occurring truncated trkB receptors have dominant inhibitory effects on brain-derived neurotrophic factor signaling. J Neurosci 1996; 16(10): 3123–3129.
23. Guillot M, Gunn-Moore FJ, Glass DJ et al. Naturally occurring tyrosine kinase inserts block high affinity binding of phospholipase C gamma and Shc to TrkC and neurotrophin-3 signaling. J Biol Chem 1995; 270(35): 20384–20390.
24. Amato A, Sartore-Bianchi A, Siena S. NTRK gene fusions as novel targets of cancer therapy across multiple tumour types. ESMO Open 2016; 1(2): e000023.
25. Nakagawa A. Trk receptor tyrosine kinases: a bridge between cancer and neural development. Cancer Lett 2001; 169(2): 107–114.
26. Barbacid M, Lamballe F, Pulido D et al. The trk family of tyrosine protein kinase receptors. Biochim Biophys Acta 1991; 1072(2–3): 113–127.
27. Vaishnavi A, Le AT, Doebele RC. TRKing down an old oncogene in a new era of targeted therapy. Cancer Discov 2015; 5(1): 25–34.
28. Crowley C, Spencer SD, Nishimura MC et al. Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. Cell 1994; 76(6): 1001–1011.
29. Smeyne RJ, Klein R, Schnapp A et al. Severe sensory and sympathetic neuropathies in mice carrying a disrupted Trk/NGF receptor gene. Nature 1994; 368(6468): 246–249.
30. Finarias I, Yoshida CK, Backus C et al. Lack of neurotrophin-3 results in death of spinal sensory neurons and premature differentiation of their precursors. Neurosci 1996; 17(6): 1065–1078.
31. Huang EJ, Reichardt LF. Neurotrophins: roles in neural development and function. Annu Rev Neurosci 2001; 24: 677–736.
32. Chen KS, Nishimura MC, Armanini MP et al. Disruption of a single allele of the nerve growth factor gene results in atrophy of basal forebrain cholinergic neurons and memory deficits. J Neurosci 1997; 17(19): 7288–7296.
33. Kermani P, Hempstead B. Brain-derived neurotrophic factor: a newly described mediator of angiogenesis. Trends Cardiovasc Med 2007; 17(4): 140–143.
34. Kermani P, Rafii D, Jin DK et al. Neurotrophins promote revascularization by local recruitment of TrkB+ endothelial cells and systemic mobilization of hematopoietic progenitors. J Clin Invest 2003; 113(5): 653–663.
35. Dissen GA, Hill DF, Costa ME et al. A role for trkA nerve growth factor receptors in mammalian ovulation. Endocrinology 1996; 137(1): 198–209.
36. Coppola V, Barrick CA, Southon EA et al. Ablation of TrkA function in the immune system causes B cell abnormalities. Development 2004; 131(20): 5185–5195.
37. Greco A, Villa R, Fusetti L et al. The Gly571Arg mutation, associated with the autonomic and sensory disorder congenital insensitivity to pain with anhidrosis, causes the inactivation of the NTRK1/nerve growth factor receptor. J Cell Physiol 2000; 182(1): 127–133.
38. Indu Y, Tsuruta M, Hayashida Y et al. Mutations in the TRKA/NGF receptor gene in patients with congenital insensitivity to pain with anhidrosis. Nat Genet 1996; 13(4): 485–488.
39. Klein R, Smeyne RJ, Wurst W et al. Targeted disruption of the trkB neurotrophin receptor gene results in nervous system lesions and neonatal death. Cell 1993; 75(1): 113–122.
40. Xi B, Goulding EH, Zang K et al. Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. Nat Neurosci 2003; 6(7): 736–742.
41. Yeo GSH, Connie Huang C-C, Rochford J et al. A de novo mutation affecting human TrkB associated with severe obesity and developmental delay. Nat Neurosci 2004; 7(11): 1187–1189.
42. Martin-Zanca D, Hughes SH, Barbacid M. A human oncogene formed by the fusion of truncated tropomyosin and protein tyrosine kinase sequences. Nature 1986; 319(6056): 743–748.
43. Ardini E, Bosotti R, Borgia AL et al. The TPM3-NTRK1 rearrangement is a recurring event in colorectal carcinoma and is associated with tumor sensitivity to TRKA kinase inhibition. Mol Oncol 2014; 8(8): 1495–1507.
44. Sartore-Bianchi A, Ardini E, Borgia AL et al. Sensitivity to entrectinib of a novel LMNA-NTRK1 gene fusion responsive to crizotinib. J Natl Cancer Inst 2016; 108(1): djv307.
45. Wong V, Pavlick D, Brennan T et al. Evaluation of a congenital infantile fibrosarcoma by comprehensive genomic profiling reveals an LMNA-NTRK1 gene fusion responsive to crizotinib. J Natl Cancer Inst 2016; 108(1): djv307.
46. Church AJ, Calicchio ML, Nardi V et al. Recurrent EML4-NTRK3 fusions in infantile fibrosarcoma and congenital mesoblastic nephroma suggest a revised testing strategy. Mod Pathol 2018; 31(3): 463–473.
47. Lakhani SR, Ellis IO, Schnitt SJ et al. WHO Classification of Tumours of the Breast. Geneva: World Health Organization 2012.
48. Tognon C, Knezevich SR, Huntsman D et al. Expression of the ETV6-NTRK3 gene fusion as a primary event in human secretory breast carcinoma. Cancer Cell 2002; 2(5): 367–376.
49. Scutara V, Vaneeck T, Sima R et al. Mammary analogue secretory carcinoma of salivary glands, containing the ETV6-NTRK3 fusion gene: a hitherto undescribed salivary gland tumor entity. Am J Surg Pathol 2010; 34(5): 599–608.
50. Anderson JL, Haidar YM, Armstrong WB et al. Analysis of clinical features of mammary analogue secretory carcinoma using the surveillance, epidemiology, and end results database. JAMA Otolaryngol Head Neck Surg 2019; 145(1): 91–93.
51. Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. Cell 2014; 159(3): 676–690.
52. Bongarzone I, Pierotti MA, Monzini N et al. High frequency of activation of tyrosine kinase oncogenes in human papillary thyroid carcinoma. Oncogene 1989; 4(12): 1457–1462.
53. Greco A, Pierotti MA, Bongarzone I et al. TRK-T1 is a novel oncogene formed by the fusion of TPR and TRK genes in human papillary thyroid carcinomas. Oncogene 1992; 7(2): 237–242.
54. Waiwajwalku W, Nakamura S, Hasegawa Y et al. Low frequency of rearrangements of the ret and trk proto-oncogenes in Japanese thyroid papillary carcinomas. Jpn J Cancer Res 1992; 83(7): 671–675.
55. Saed S, Schlumberger M, Suarez HG. Oncogenes and anti-oncogenes in human epithelial thyroid tumors. J Endocrinol Invest 1994; 17(5): 371–379.
56. Buriti MG, Bongarzone I, Ferraresi G et al. A sequence analysis of the genomic regions involved in the rearrangements between TPM3 and NTRK1 genes producing TRK oncogenes in papillary thyroid carcinomas. Genomics 1995; 28(1): 15–24.
57. Devlinvourt C, Patey M, Flament JB et al. Ret and trk proto-oncogene activation in thyroid papillary carcinomas in French patients from the Champagne-Ardenne region. Clin Biochem 1996; 29(3): 267–271.
58. Bounacer A, Schlumberger M, Wicker R et al. Search for NTRK1 proto-oncogene rearrangements in human thyroid tumours originated after therapeutic radiation. Br J Cancer 2000; 82(2): 308–314.
59. Richarte-Filho JC, Li S, Garcia-Rendueles ME et al. Identification of kinase fusion oncogenes in post-Chernobyl radiation-induced thyroid cancers. J Clin Invest 2013; 123(11): 4935–4944.
60. Leeman-Neill RJ, Kelly LM, Liu P et al. ETV6-NTRK3 is a common chromosomal rearrangement in radiation-associated thyroid cancer. Cancer 2014; 120(6): 799–807.
61. Kloosterman WP, Coebergh van den Braak RJJ, Pieterse M et al. A systematic analysis of oncogenic gene fusions in primary colon cancer. Cancer Res 2017; 77(14): 3814–3822.
72. Creancier L, Vandenberghie I, Gomes B et al. Chromosomal rearrangements involving the NTRK1 gene in colorectal carcinoma. Cancer Lett 2015; 365(1): 107–111.

73. Hechtman JF, Benayed R, Hyman DM et al. Pan-Trk immunohistochemistry is an efficient and reliable screen for the detection of NTRK fusions. Am J Surg Pathol 2017; 41(11): 1547–1551.

74. Clifton K, Raymond VM, Dasari A et al. Actionable fusions in colorectal cancer using a cell-free circulating tumor DNA (ctDNA) assay. J Clin Oncol 2018; 36(Suppl 15): 3507.

75. Rankin A, Klemper SJ, Erlich R et al. Broad detection of alterations predicted to confer lack of benefit from EGFR antibodies or sensitivity to targeted therapy in advanced colorectal cancer. Oncologist 2016; 21(11): 1306–1314.

76. Pietrantonio F, Di Nicolantonio F, Schrock AB et al. ALK, ROS1, and NTRK rearrangements in metastatic colorectal cancer. J Natl Cancer Inst 2017; 109(12): djx069.

77. Deihimi S, Lev A, Siliker M et al. BRCA2, EGFR, and NTRK mutations in mismatch repair-deficient colorectal cancers with MSH2 or MLH1 mutations. Oncotarget 2017; 8(25): 39945–39962.

78. Braghiri M, Nash GM, Morris M et al. Genomic profiling and efficacy of anti-EGFR therapy in appendiceal adenocarcinoma. J Clin Oncol 2016; 34(Suppl 4): 574.

79. Molina JR, Yang P, Cassivi SD et al. Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. Mayo Clin Proc 2008; 83(5): 584–594.

80. Vaishnavi A, Capelletti M, Le AT et al. Oncogenic and drug-sensitive NTRK1 rearrangements in lung cancer. Nat Med 2013; 19(11): 1469–1472.

81. Farago AF, Le LP, Zheng Z et al. Durable clinical response to entrectinib in NTRK1-rearranged non-small cell lung cancer. J Thorac Oncol 2015; 10(12): 1670–1674.

82. Farago AF, Taylor MS, Doebelle RC et al. Clinicopathologic features of non-small cell lung cancer harboring an NTRK gene fusion. JCO Precis Oncol 2018; (2): 1–12.

83. Doebelle RC, Davis LE, Vaishnavi A et al. An oncogenic NTRK fusion in a patient with soft-tissue sarcoma with response to the tropomyosin-related kinase inhibitor LOXO-101. Cancer Discov 2015; 5(10): 1049–1057.

84. Haller F, Knopf J, Ackermann A et al. Paediatric and adult soft tissue sarcomas with NTRK1 gene fusions: a subset of spindle cell sarcomas unified by a prominent myopericytic/haemangiopericytic pattern. J Pathol 2016; 238(5): 700–710.

85. Chiang S, Cotzia P, Hyman DM et al. NTRK fusions define a novel uterine sarcoma subtype with features of fibrosarcoma. Am J Surg Pathol 2018; 42(6): 791–798.

86. Agaram NP, Zhang L, Sung YS et al. Recurrent NTRK1 gene fusions define a novel subset of locally aggressive lipofibrosarcoma-like neural tumors. Am J Surg Pathol 2016; 40(10): 1407–1416.

87. Pavlick D, Schrock AB, Malicki D et al. Identification of NTRK fusions in pediatric mesenchymal tumors. Pediatr Blood Cancer 2017; 64(8): 791–798.

88. Alassiri AH, Ali RH, Shen Y et al. ETV6-NTRK3 as a gene fusion involved in GST: J Pathol 2016; 238(4): 543–549.

89. Shi E, Chimielecki J, Tang CM et al. FGFR1 and NTRK3 actionable alterations in “wild-type” gastrointestinal stromal tumors. J Transl Med 2016; 14(1): 339.

90. Fishvain MJ, Rolfo CD, Liu SV et al. Clinical benefit of entrectinib for patients with metastatic pancreatic cancer who harbor NTRK and ROS1 fusions. J Clin Oncol 2018; 36(Suppl 4): 521.

91. Andreasen S, Skalova A, Agaimy A et al. ETV6-NTRK3 as a gene fusion involved in GST: J Pathol 2016; 238(4): 543–549.

92. Sigal D, Tartar M, Xavier M et al. Activity of entrectinib in a patient with the first reported NTRK fusion in neuroendocrine cancer. J Natl Compr Canc Netw 2017; 15(11): 1317–1322.

93. Roberts KG, Li Y, Payne-Turner D et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med 2014; 371(11): 1005–1015.

94. Sugihara A, Shi E, Chmielecki J et al. NTRK3 kinase fusions in Spitz tumours. Nat Commun 2014; 5: 3116.

95. Drilon A, Nagasubramanian R, Blake JF et al. A next-generation TRK kinase inhibitor overcomes acquired resistance to prior TRK kinase in colorectal cancer cell lines unveils clinically actionable kinase targets. Nat Comms 2015; 6(1): 7002.

96. Lee SJ, Li GG, Kim ST et al. NTRK1 rearrangement in colorectal cancer discov 2017; 7(9): 963–972.

97. Lee SJ, Li GG, Kim ST et al. NTRK1 rearrangement in colorectal cancer discov 2017; 7(9): 963–972.

98. Eguchi M, Eguchi-Ishimae M, Tojo A et al. Fusion of ETV6 to NTRK1 in acute myeloid leukemia with t(12;37) and NTRK1 rearrangement in colorectal cancer using a cell-free circulating tumor DNA (ctDNA) assay. J Clin Oncol 2015; 1049–1057.

99. Eguchi M, Eguchi-Ishimae M, Tojo A et al. Fusion of ETV6 to NTRK1 in acute myeloid leukemia with t(12;37) and NTRK1 rearrangement in colorectal cancer using a cell-free circulating tumor DNA (ctDNA) assay. J Clin Oncol 2015; 1049–1057.

100. Wiesner T, He J, Yelensky R et al. Kinase fusions are frequent driver genomic alterations in glioblastoma. Nat Genet 2013; 45(10): 1049–1057.

101. Drilon A, Siena S, Ou SI et al. Safety and antitumor activity of the multitargeted pan-TRK, ROS1, and ALK inhibitor entrectinib: combined results from two phase I trials (ALKA-372-001 and STARTK1-1). Cancer Discov 2017; 7(4): 400–409.

102. Wu G, Diaz AK, Paugh BS et al. The genomic landscape of diffuse neuroendocrine cancer cell line unveils clinically actionable kinase targets. Nat Comms 2015; 6(1): 7002.

103. Lee SJ, Li GG, Kim ST et al. NTRK1 rearrangement in colorectal cancer discov 2017; 7(9): 963–972.

104. Shi E, Chimielecki J, Tang CM et al. FGFR1 and NTRK3 actionable alterations in “wild-type” gastrointestinal stromal tumors. J Transl Med 2016; 14(1): 339.

105. Fishvain MJ, Rolfo CD, Liu SV et al. Clinical benefit of entrectinib for patients with metastatic pancreatic cancer who harbor NTRK and ROS1 fusions. J Clin Oncol 2018; 36(Suppl 4): 521.

106. Andreasen S, Skalova A, Agaimy A et al. ETV6-NTRK3 as a gene fusion involved in GST: J Pathol 2016; 238(4): 543–549.

107. Sigal D, Tartar M, Xavier M et al. Activity of entrectinib in a patient with the first reported NTRK fusion in neuroendocrine cancer. J Natl Compr Canc Netw 2017; 15(11): 1317–1322.

108. Roberts KG, Li Y, Payne-Turner D et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med 2014; 371(11): 1005–1015.

109. Sugihara A, Shi E, Chmielecki J et al. NTRK3 kinase fusions in Spitz tumours. Nat Commun 2014; 5: 3116.

110. Lee SJ, Li GG, Kim ST et al. NTRK1 rearrangement in colorectal cancer discov 2017; 7(9): 963–972.

111. Medico E, Russo M, Picco G et al. The molecular landscape of colorectal cancer cell line unveils clinically actionable kinase targets. Nat Comms 2015; 6(1): 7002.

112. Lee SJ, Li GG, Kim ST et al. NTRK1 rearrangement in colorectal cancer discov 2017; 7(9): 963–972.
analysis of STARTRK-2, STARTRK-1 and ALKA-372-001. In ESMO 2018 Congress, Munich, Germany, LBA17, 2018.

116. Lassen UN, Albert CM, Kummar S et al. Larotrectinib efficacy and safety in TRK fusion cancer: an expanded clinical dataset showing consistency in an age and tumor agnostic approach.Ann Oncol 2018; 29(Suppl 8): viii133–viii148.

117. Hong DS, Bauer TM, Lee JJ et al. Larotrectinib in adult patients with solid tumours: a multi-centre, open-label, phase I dose-escalation study. Ann Oncol 2019; 30(2): 325–331.

118. Russo M, Misale S, Wei G et al. Acquired resistance to the TRK inhibitor entrectinib in colorectal cancer. Cancer Discov 2016; 6(1): 36–44.

119. Okamura R, Boichard A, Kato S et al. Analysis of NTRK alterations in pan-cancer adult and pediatric malignancies: implications for NTRK-targeted therapeutics. JCO Precis Oncol 2018; 2(1): 1.

120. Nanda N, Fennell T, Low JA. Identification of tropomyosin kinase receptor (TRK) mutations in cancer. J Clin Oncol 2015; 33(Suppl 15): 1553.