

TRKing Down an Old Oncogene in a New Era of Targeted Therapy

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ABSTRACT

The use of high-throughput next-generation sequencing techniques in multiple tumor types during the last few years has identified *NTRK1*, 2, and 3 gene rearrangements encoding novel oncogenic fusions in 19 different tumor types to date. These recent developments have led us to revisit an old oncogene, *Trk* (originally identified as *OncD*), which encodes the *TPM3-NTRK1* gene fusion and was one of the first transforming chromosomal rearrangements identified 32 years ago. However, no drug has yet been approved by the FDA for cancers harboring this oncogene. This review will discuss the biology of the TRK family of receptors, their role in human cancer, the types of oncogenic alterations, and drugs that are currently in development for this family of oncogene targets.

Significance: Precision oncology approaches have accelerated recently due to advancements in our ability to detect oncogenic mutations in tumor samples. Oncogenic alterations, most commonly gene fusions, have now been detected for the genes encoding the TRKA, TRKB, and TRKC receptor tyrosine kinases across multiple tumor types. The scientific rationale for the targeting of the TRK oncogene family will be discussed here. *Cancer Discov*; 5(1); 25-34. ©2014 AACR.

INTRODUCTION

The identification of dominant oncogenic mutations and our ability to specifically inhibit these genetic abnormalities with targeted inhibitors have altered the therapeutic approach for many patients with cancer, particularly those with non-small cell lung cancer (NSCLC). Activating point mutations, in-frame insertions/deletions, gene amplification, and gene rearrangements can serve as predictive biomarkers for oncogene-targeted therapies and thus help select patients that have a high likelihood of benefiting from a particular therapy. There are currently two well-established paradigms of this targeted therapy approach in NSCLC, both of which highlight the potential success of this strategy for other oncogene targets. Epidermal growth factor receptor (*EGFR*) mutation-positive NSCLC patients (comprising ~18% of lung adenocarcinomas) and anaplastic lymphoma kinase (*ALK*) gene rearrangement-positive NSCLC patients (encompassing ~5% of lung adenocarcinomas) respond significantly better to the targeted

therapies erlotinib and crizotinib, respectively, compared with the standard-of-care chemotherapy (1). *EGFR* mutation-positive patients who are treated with an *EGFR* tyrosine kinase inhibitor (TKI) have an objective response rate (ORR) of about 70% and a progression-free survival (PFS) time of approximately 10 months, both of which are superior to chemotherapy (2). *ALK* gene rearrangement-positive patients showed a response rate of approximately 65% and a PFS of approximately 8 months when treated with crizotinib, also superior to chemotherapy (3).

The paradigm of cancer treatment is shifting toward precision oncology. In this model, patients are selected for therapy using predictive biomarkers, such as oncogenic mutations, rather than using empiric chemotherapy. Many of the actionable or potentially actionable oncogenes that represent molecular subtypes in NSCLC involve genomic rearrangements with genes encoding receptor tyrosine kinases (RTK), such as *ALK*, *ROS1*, *RET*, and most recently *NTRK1* (4-7). The unprecedented improvement in patient outcomes with oncogene-targeted therapies suggests that even rare oncogenes, such as *ROS1* gene rearrangements (which occur at a frequency of ~1%-2%), should be investigated as therapeutic targets, as this molecular subset represents approximately 2,500 patients in the United States each year (8, 9). Indeed, a recent study of crizotinib in patients with *ROS1*-positive NSCLC highlights the ability to successfully accrue rare oncogene subtypes (10). The study of these low-frequency oncogenes not only applies to NSCLC, but also is directly relevant to the treatment of numerous other cancer types: *ALK*, *ROS1*, *RET*, and *NTRK1* gene rearrangements have also been observed in other malignancies,

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KEY CONCEPTS

- *NTRK1* gene fusions were first identified in colon cancer in 1982, but have since been identified in multiple tumor types in recent years; gene fusions involving the highly homologous *NTRK2* and *NTRK3* genes have also been identified in 11 different tumor types.
- Drugs with activity against the TRK family of receptor tyrosine kinases are currently in development for patients with oncogenic alterations in *NTRK1*, 2, and 3.

expanding the relevance of this work to colorectal cancer, thyroid cancer, cholangiocarcinoma, glioblastoma, inflammatory myofibroblastic tumors (IMT), ovarian cancer, bladder cancer, sarcomas, and others (11–17). Indeed, isolated reports show the success of targeting oncogenes across multiple tumor types (15, 18). It was estimated in 2007 that gene fusions were reported in approximately 20% of all cancers, accounting for a significant proportion of cancer morbidity and mortality (19). The emergence of high-throughput genomic technologies and programmatic sequencing efforts, such as the NCI/National Human Genome Research Institute Cancer Genome Atlas Network and the Sanger Cancer Genome Project, have generated the molecular profiles of numerous cancers, and this emergent technology has enabled the identification of many additional gene fusions that are putative oncogenes and predicted to be conserved as drivers across breast cancer, glioblastoma, lung cancer, colorectal cancer, and other tumors (16, 17, 20–22). This review describes the emergence of an increasingly described class of potential oncogene targets in cancer, the TRK family of kinases.

TRK FAMILY BIOLOGY

The *NTRK1* gene encodes TRKA, which is a member of the TRK (tropomyosin-receptor kinase) family of RTKs that includes TRKB (encoded by *NTRK2*) and TRKC (encoded by *NTRK3*) (12, 23). TRKA, TRKB, and TRKC play important roles in nervous system development through their regulation of cell proliferation, differentiation, apoptosis, and survival of neurons in both the central and peripheral nervous systems. The TRK receptors are expressed abundantly in the nervous system, as well as in many other nonneuronal cell types and tissues, including monocytes, the lung, bone, and pancreatic beta cells (24). TRKA, TRKB, and TRKC are most frequently activated by their primary ligands nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin 3 (NT-3), respectively (25, 26). However, overexpression studies in cell lines suggest the possibility of promiscuity among these neurotrophin ligands and all three receptors that may be cell-type and neurotrophin concentration dependent (23). Additional studies have shown that NT-3 may activate TRKA and TRKB, and NT-4/5 may activate TRKB (27). The binding of each of these ligands to its cognate receptor, such as the binding of NGF to TRKA, induces receptor homodimerization and transphosphorylation of five critical tyrosine (Y) residues (Y496, Y676, Y680,

Y681, and Y791). Y496 and Y791 serve as phosphorylation-dependent binding sites for various adaptor proteins that contain SH2 or PTB domains, primarily SHC1, PLC γ , and GAB1, but others include FRS2, GRB2, IRS1, IRS2, and SH2B (Fig. 1; refs. 12, 28–30). Evidence from several studies points to activation of the PI3K signaling pathway by RAS or GAB1, although it may also be activated from other mechanisms (29, 31–33). Once activated, the three wild-type TRK family members most frequently signal through several downstream signaling pathways, including SHC–RAS–MAPK, PI3K–AKT, or PLC γ –PKC, depending on which docking protein(s) bind to the critical phosphorylated tyrosines Y496 and Y791 (34). Activation of these signaling cascades results in transcriptional and other cell programs that mediate cellular proliferation, synaptic plasticity, neurite outgrowth and repair, prevention or repair of neurodegeneration, sensory neuron maintenance, or apoptosis (12, 34–37).

It is expected that most TRK fusions would use many or all of the same downstream signaling cascades as the full-length receptors, given the preservation of the kinase domain and the critical tyrosine docking sites; however, this is a relatively unexplored area compared with decades of detailed studies on the signaling mechanisms used by the full-length receptors, particularly in the rat pheochromocytoma cell line PC12 (Fig. 1). The *ETV6–NTRK3* fusion might be an exception, because it lacks the critical Y485 docking site for the preferential adaptor SHC1 due to the location of the breakpoint in the fusion, and evidence points to the use of an alternate adaptor, IRS-1 (38). Cell-type context and differential subcellular localization of fusions might alter the signaling program of the oncogenic fusion kinases.

Studies of TRKA fusions in thyroid cancer have revealed the TRK oncogenes (TRK, TRK-T1-T3) are capable of binding a number of different adaptor molecules, similar to full-length TRKA, but are predominantly engaged in signaling through the RAS–RAF–MAPK pathway (Fig. 1; refs. 39–41). The STAT3 signaling pathway was identified as playing a role in NIH-3T3 transformation by TRK oncogenes (42). Interestingly, the constitutive signaling induced by TRK oncogenes has also been shown to result in neuronal differentiation of PC12 cells (43). It was also elegantly demonstrated that the TRK oncogenes are capable of transforming not just NIH-3T3 fibroblasts, the commonly used model system for studies of oncogenic transformation, but also a more relevant *in vivo* model of cellular transformation, thyroid epithelial cells (44). Similarly, *in vivo* transformation of mammary epithelia was shown using the *ETV6–NTRK3* fusion, clearly demonstrating the potency of these oncogenes in multiple model systems (45). Studies of TRKA fusion signaling in endogenous colorectal (KM12) and lung cancer (CUTO-3) cells have been conducted recently by our laboratory. The *TPM3–NTRK1*, *MPRIP–NTRK1*, and *CD74–NTRK1* fusions seem to signal predominantly through the SHC–RAS–MAPK pathway in endogenous colorectal and lung cancer cell lines, but can engage PI3K–AKT or STAT3 signaling in certain cell types (4). Studies of the *ETV6–NTRK3* fusion have demonstrated that oncogenic signaling is engaged through IRS-1, but due to the limited availability of cell lines expressing the *ETV6–NTRK3* fusion, most studies were conducted using

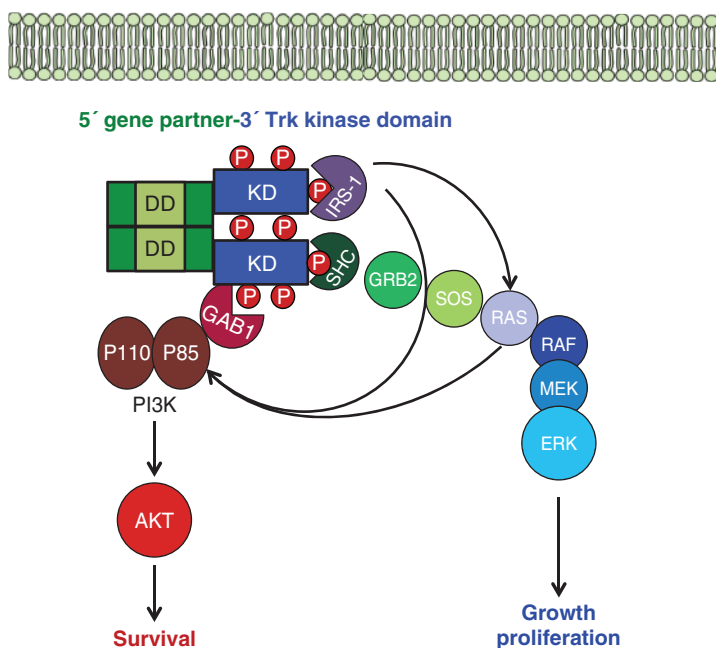


Figure 1. TRK fusion signaling. Schematic showing common signaling mechanisms for an example of a cytoplasmic (nonmembrane bound) chimeric TRK gene fusion is shown. Gene fusions are constitutively activated, or phosphorylated, often as a result of dimerization mediated by sequences in the 5' gene. SH2 and PTB domain containing adaptors compete for binding at specific tyrosine residues, which most frequently results in propagation of the downstream signaling pathways shown. DD, dimerization domain; KD, kinase domain.

a cDNA of the fusion expressed in various cell lines, such as fibroblasts, resulting predominantly in activation of the RAS-MAPK signaling pathway, but also PI3K-AKT, often simultaneously (Fig. 1; ref. 38). The potential for simultaneous, dual activation of multiple downstream pathways may result in a potent oncogene, as this enables the activation of both proliferative and antiapoptotic pathways (46). Studies by the same group have also demonstrated a critical role for upstream RTK signaling, through the insulin-like growth factor receptor (IGF1R) in *ETV6-NTRK3* fusion-driven tumorigenesis (38). Studies in mice in selected *NTRK1* and *NTRK3* fusions have suggested these fusions likely play an important, early role in tumor progression (45, 47). Although P75^{NTR} can modulate the activity and signaling of the full-length TRK receptors, its interaction with oncogenic TRK fusions has not been studied to date (23).

TRK Family in Cancer

Mutations in TRK family members have been reported in numerous malignancies, including ovarian cancer, colorectal cancer, melanoma, and lung cancer, but among *NTRK1* alterations other than gene fusions, only an in-frame deletion of *NTRK1* (Δ TRKA) in acute myeloid leukemia (AML) and a splice variant of *NTRK1* (TRKAIII) in neuroblastoma have been functionally characterized as oncogenic to date (Fig. 2A; refs. 48–54). The deletion in Δ TRKA results in the loss of 75 amino acids in the extracellular domain of TRKA, removes four glycosylation sites adjacent to the transmembrane domain, and transforms both fibroblasts and epithelial cells (53). The TRKAIII splice variant, which was identified in a neuroblastoma cell line, results in the loss of exons 6, 7, and 9, and the corresponding loss of the extracellular domain Ig-like C2-type I (IG-C2) as well as multiple glycosylation sites (51). TRKAIII is constitutively active (ligand-independent), and its expression is promoted by hypoxic conditions (51). Although not yet identified in human tumor samples to date, mutations

in the extracellular domain of TRKA, P203A and C345S, have both been characterized as transforming (55, 56). These studies may point to regions of interest where mutations have been identified in human tumors; for example, a relative cluster of mutations occurs in *NTRK1* at the R342 position in close proximity to the C345 site identified by mutagenesis (57). The most common mechanism of oncogenic activation of TRKA is through genomic rearrangement and the creation of a gene fusion (12). Interestingly, all of these different mechanisms of oncogenic activation of TRKA (gene rearrangements, deletion, and splice variant) contain the loss of some of the extracellular domain of TRKA. The loss of these common sequences suggests the presence of critical regulatory domains in the extracellular domain of TRKA (and potentially B and C) that, when lost, results in constitutive activation of the kinase domain and thus its oncogenic capacity and is supported by mutagenesis studies in which Ig-like domains in the extracellular region of TRKA were deleted (58).

Autocrine and paracrine signaling by TRK receptors have been implicated as protumorigenic in several different tumor types. An autocrine loop involving TRKA and NGF is associated with protumorigenic activity in both breast and prostate carcinomas; similarly, TRKB and BDNF have been shown to play a protumorigenic role in several malignancies, including both breast and prostate cancers (59, 60). TRKB signaling has also been shown to promote anoikis resistance and induce metastatic programs in numerous cancers (61). Expression of TRKA and TRKC wild-type receptors is associated with a positive prognosis in patients with neuroblastoma (excluding expression of the splice variant TRKAIII), whereas TRKB expression is correlated with a poorer prognosis (62, 63).

TRK Family Oncogenic Fusions

The typical gene structure for an oncogenic fusion is that the 3' region of a proto-oncogene (encoding the kinase

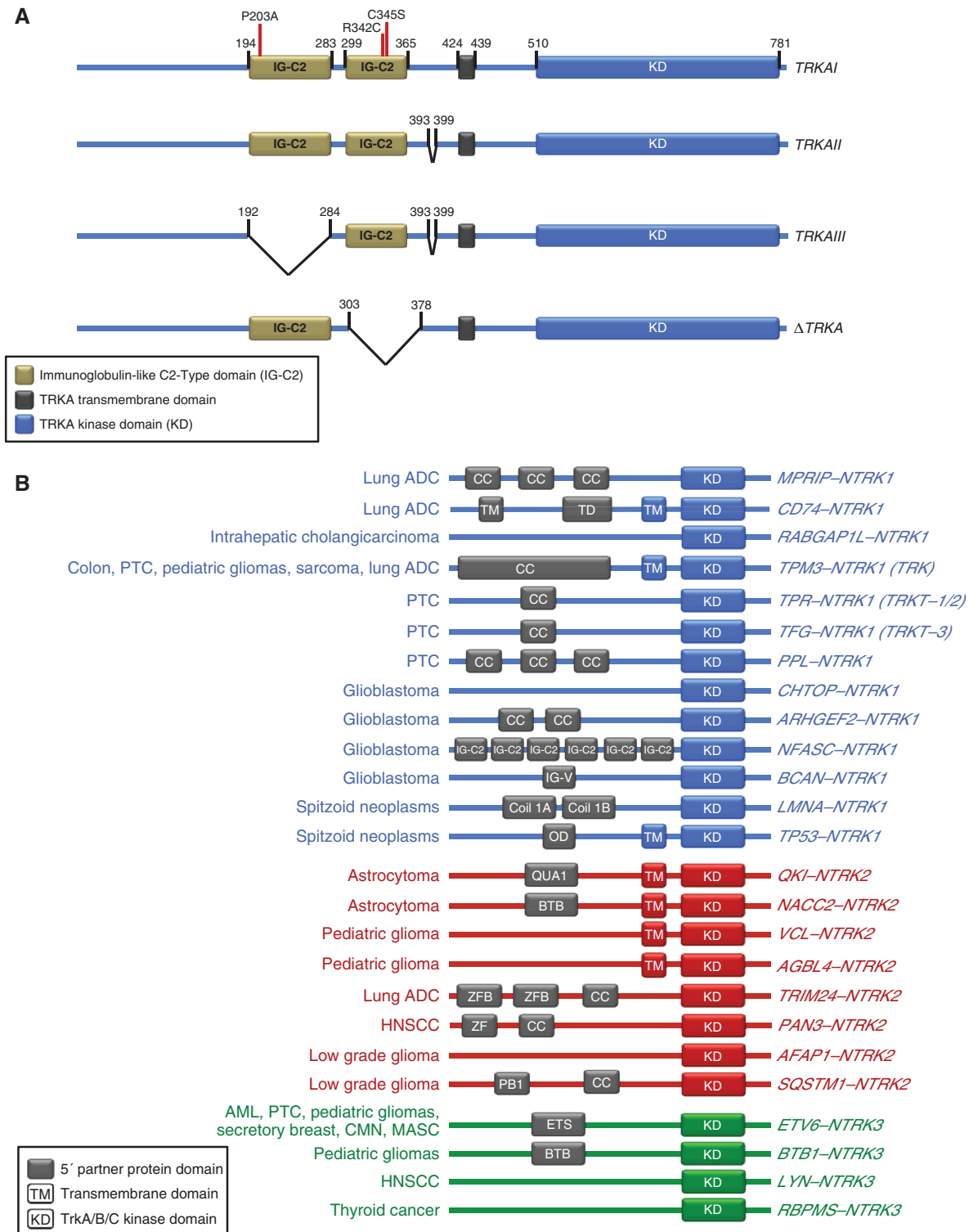


Figure 2. TRKA oncogenic variants and TRK gene fusion partners. **A**, schematic of TRKA isoforms, deletions, and mutations are shown. Mutations are shown in the TRKAI isoform. Amino acid position numbers are shown in black. **B**, schematic showing the known *NTRK1* (blue), *NTRK2* (red), and *NTRK3* (green) fusions and the tumor types in which they have been identified. It is important to note that not all of these gene fusions have yet been characterized functionally, but each one occurred in-frame with an intact TRK kinase domain and are thus potentially oncogenic. Known or potential 5' dimerization domains are shown (gray), and 3' domains shown in blue (*NTRK1*), red (*NTRK2*), or green (*NTRK3*). No protein domains are shown for fusions that lack a reported breakpoint. Fusion proteins are not drawn to scale. ADC, adenocarcinoma; PTC, papillary thyroid cancer; AML, acute myeloid leukemia; CMN, congenital mesoblastic nephroma; MASC, mammary analogue secretory carcinoma; HNSCC, head and neck squamous cell cancer; CCD, coiled-coil domain; TD, trimerization domain; IG-C2, Immunoglobulin-like C2-type domain; IG-V, Immunoglobulin-like V domain; OD, oligomerization domain; ZF QUA1, Quaking 1 domain; BTB, bric-a-brac, tramtrack, and broad complex domain; ETS, E26 transformation-specific domain.

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domain) is juxtaposed to 5' sequences from an unrelated gene via an intra- or interchromosomal rearrangement. The resultant novel oncogene is both aberrantly expressed and has constitutive activation of the kinase domain. In 1982, the same year that the *BCR* and *ABL* genes were implicated in the first oncogenic translocation on the Philadelphia chromosome in chronic myelogenous leukemia (CML), the first *NTRK1* gene fusion was identified in a colon cancer sample and contained sequences from *TPM3* (nonmuscle tropomyosin; refs. 64, 65). The incidence and therapeutic potential of *TPM3-NTRK1* in colorectal cancer was recently revisited after 32 years by Ardini and colleagues (22), reaffirming that this *NTRK1* fusion is indeed a recurrent, albeit infrequent, oncogene in colon cancer. Each of the colorectal cases harboring *NTRK1* fusions identified thus far express the *TPM3-NTRK1* oncogene, suggesting a preference for *TPM3* as the partner gene in this particular tissue, similar to *EML4* with *ALK* in lung cancer (4, 11, 16, 22, 64). In addition, *TRKC* and very recently *TRKB* have also been shown to form oncogenic chimeras in multiple tumor types (Fig. 2B; refs. 66, 67). The *ETV6-NTRK3* fusion has been identified as the dominant oncogene in several malignancies, including secretory breast carcinoma, mammary analogue secretory carcinoma (MASC) of the salivary gland, congenital fibrosarcoma, congenital mesoblastic nephroma, AML, and radiation-associated papillary thyroid cancer (PTC; refs. 45, 67-74). *ETV6-NTRK3* fusions can vary slightly with regard to the breakpoint in different cancer types, but always retain the SAM dimerization domain from *ETV6* and the kinase domain of *TRKC*. Chromosomal rearrangements have been observed between *NTRK1* and *TFG*, *TPM3*, or *TPR* in PTC, the most common malignancy of the thyroid (12). Interestingly, many of these activating 5' gene fusion partners are promiscuous among various kinase fusion classes (12, 16). Although most of the *NTRK1*, *NTRK2*, and *NTRK3* fusions identified thus far fit the paradigm and contain a 5' gene partner with a dimerization domain, several of the partners do not contain clearly identifiable dimerization domains (Fig. 2B). 5' Gene partners often contain one or more dimerization domains, such as the prototypical coiled-coil domain(s), and the corresponding constitutive tyrosine kinase activity that occurs results in uninterrupted downstream signaling messages for the cell to proliferate aberrantly and survive (12). Oncogenic gene rearrangements involving *ROS1* may be an exception to this paradigm, as many of the 5' gene partners have no known dimerization domains, but have still been shown to possess transforming properties (16). One can speculate based on this that activation of *ROS1* may only require loss of 5' sequences that act as an autoinhibitory signal in the full-length RTK. A similar mechanism may be worth investigating in TRK family fusions. For example, *VCL-NTRK2* has not been functionally characterized for transforming properties, but may be activated without a 5' dimerization domain through the loss of a regulatory domain or by a different unknown mechanism, similar to many *ROS1* fusions.

NGS Identification of *NTRK1*, 2, and 3 Fusions

In the last year, many next-generation sequencing (NGS) efforts, including programmatic, disease-oriented whole-genome, and/or transcriptome projects and also targeted

clinical NGS platforms have resulted in the identification of *NTRK* family fusions in numerous tumor types. *NTRK1* fusions were recently identified in lung adenocarcinoma, intrahepatic cholangiocarcinoma, spitzoid neoplasms, glioblastoma, and pontine glioma (4, 75-79). These findings were further validated in lung adenocarcinoma, as well as the discovery of novel fusions in PTC, and glioblastoma using a novel, targeted technique known as anchored multiplex PCR (80). It is also important to note that although TRK fusions were not detected initially by The Cancer Genome Atlas (TCGA), revisiting these data in 20 different cancers with a more efficient computational pipeline for the detection of gene fusions identified TRK fusions in eight additional tumor types (Table 1; refs. 9, 17). The first evidence of gene fusions involving the *NTRK2* gene came in pilocytic astrocytoma and very soon afterward in pontine glioma (66, 74, 75). New tumor types with *NTRK3* fusions were also identified, including PTC, pontine glioma, and Philadelphia chromosome-like acute lymphoblastic leukemia (Ph-ALL; refs. 68, 75, 81). Each of the three *NTRK* family genes can rearrange with multiple 5' gene partners (Fig. 2).

A unique biologic aspect of the *ETV6-NTRK3* fusion is that it was the first oncogenic gene fusion to be identified in numerous different cancer tumor tissues. In each of those different tumor types, all of which are relatively rare malignancies, *ETV6-NTRK3* is the dominant oncogene. For example, 100% of MASC of salivary glands and 93% of secretory breast cancers harbor *ETV6-NTRK3* fusions (45, 70). This observation is similar to CML, where *BCR-ABL* is found in the vast majority of cases. However, in most tumors in which TRK fusions are identified, they represent only a small proportion of patients (Table 1). Collectively, the TRK family represents a sizeable number of cases distributed across multiple tumor types.

TRK INHIBITORS

Given the long history of oncogenic TRK alterations, one might ask why it has taken so long to develop drugs for this target in cancer. Several reasons likely contribute to the slow development of this target, including the lack of selective inhibitors and the relative difficulty in screening large tumor cohorts when this oncogene was first identified in the early 1980s. Similar to TRK family alterations, oncogenic *ALK* gene rearrangements were found to be important in cancer long before the first *ALK* inhibitor was FDA approved. The first *ALK* gene rearrangement was identified in anaplastic large cell lymphoma in 1994, but no *ALK*-targeted therapies were developed in this disease until many years later. The critical moment for *ALK* inhibitor development came in 2007 with the discovery of *ALK* gene rearrangements in NSCLC (5). The corresponding FDA approval of crizotinib for *ALK*-positive metastatic NSCLC was exceedingly fast, taking only 4 years from the time of first identification of *ALK* rearrangements in this patient population (5). This rapid approval highlights the successful strategy of precision oncology by matching targeted therapies with biomarker-selected patients. Neurotrophins and TRK receptors, particularly *TRKA*, have been pursued in the past as drug targets for the treatment of chronic pain (35), and a few studies have pursued the

Table 1. Oncogenic TRK fusions are found across multiple tumor types

Gene fusion	Cancer	Frequency	Detection method(s)
<i>NTRK1</i>	Lung adenocarcinoma	3/91 (3.3%)	Targeted NGS (FMI), FISH ⁴
<i>NTRK1</i>	Intrahepatic cholangiocarcinoma	1/28 (3.6%)	Targeted NGS (FMI) ⁷⁷
<i>NTRK1</i>	Colorectal cancer	3 Isolated reports 1/66 (1.5%)	cDNA library, FISH, PCR ^{4, 11, 63} PCR, IHC ²²
<i>NTRK1</i>	Papillary thyroid cancer	28/228 (12.3%)	PCR ¹²
<i>NTRK1</i>	Spitzoid neoplasms	23/140 (16.4%)	Targeted NGS (FMI), FISH, IHC ⁷⁸
<i>NTRK1</i>	Glioblastoma	2/185 (1.1%) 4/162 (2.5%) 1/157	NGS ⁷⁵ NGS/PCR ⁷⁶
<i>NTRK1</i>	Sarcoma (TCGA)	1/103 (1%)	RNA-Seq ¹⁷
<i>NTRK2</i>	Astrocytoma	3/96 (3.1%)	NGS ⁶⁵
<i>NTRK2</i>	Lung adenocarcinoma (TCGA)	1/513 (0.2%)	RNA-Seq ¹⁷
<i>NTRK2</i>	Head and neck squamous cell carcinoma (TCGA)	1/411 (0.2%)	RNA-Seq ¹⁷
<i>NTRK2</i>	Brain lower grade glioma (TCGA)	2/461 (0.4%)	RNA-Seq ¹⁷
<i>NTRK3</i>	Secretory breast carcinoma	12/13 (92%)	FISH, PCR ⁴⁴
<i>NTRK3</i>	Mammary analogue secretory carcinoma	15/15 (100%)	FISH ⁶⁹
<i>NTRK3</i>	Papillary thyroid cancer	9/62 (14.5%) ^a 7/243 (2.9%) ^b	RNA-Seq ^{67, 73}
<i>NTRK3</i>	Acute myeloid leukemia	2 Case reports	PCR, FISH ^{68, 72}
<i>NTRK3</i>	Congenital mesoblastic nephroma	5/6 (83%)	PCR and FISH ⁶⁶
<i>NTRK3</i>	Congenital fibrosarcomas	10/11 (91%) 5/5 (100%)	PCR ⁷¹ PCR and FISH ⁶⁶
<i>NTRK3</i>	Ph-like acute lymphoblastic leukemia	1/154 (0.7%)	NGS ⁷⁹
<i>NTRK3</i>	Colon adenocarcinoma (TCGA)	2/286 (0.7%)	RNA-Seq ¹⁷
<i>NTRK3</i>	Thyroid carcinoma (TCGA)	7/498 (1.5%)	RNA-Seq ¹⁷
<i>NTRK3</i>	Skin cutaneous melanoma (TCGA)	1/374 (0.3%)	RNA-Seq ¹⁷
<i>NTRK3</i>	Head and neck squamous cell carcinoma (TCGA)	1/411 (0.2%)	RNA-Seq ¹⁷
<i>NTRK1/NTRK2/NTRK3</i>	Pediatric gliomas	8/112 (7.1%)	NGS ⁷⁴

NOTE: The frequency of *NTRK1* (blue), *NTRK2* (red), and *NTRK3* (green) gene fusions indicating the tumor type and the detection method that was used in each study. Only positive studies are listed, and thus the actual prevalence may be lower than reported.

^aPost-Chernobyl.

^bSporadic.

Abbreviations: FMI, Foundation Medicine, Incorporated; TCGA, The Cancer Genome Atlas.

TRK family as a therapeutic target in cancer (60, 82, 83). High levels of homology between TRKA, TRKB, and TRKC within the intracellular kinase domains have resulted in the synthesis of small-molecule inhibitors that target all three TRK family members (pan-TRK inhibitors; Table 2).

Clinical trials of TRK inhibitors will need to investigate potential side effects that may arise from inhibition of the full-length TRK receptors in normal tissues. Loss of normal regulation of TRKA, TRKB, or TRKC receptor activity can result in numerous human diseases. TRK receptors are known for mediating pain sensation and can play a role in chronic pain (35, 84). TRKA loss-of-function mutations are seen in class IV hereditary sensory and autonomic neu-

ropathies (HSAN), such as the genetic disorder congenital insensitivity to pain with anhidrosis (CIPA; refs. 36, 84). Loss-of-function mutations in TRKB result in energy imbalances, the loss of appetite control and obesity, and neuronal defects such as memory impairment (37). Similarly, loss of BDNF expression in the cerebellum of the mutant stargazer mouse is associated with a severe ataxia phenotype (85), and *Ntrk2* homozygous mutant mice have severe neurologic deficits (37), suggesting a critical role for the BDNF-TRKB signaling axis in normal neurologic development. However, it remains unclear whether inhibition of the full-length TRK receptors will produce symptoms that mimic developmental loss of signaling in this receptor family. A more detailed evaluation

Table 2. TRK inhibitors under development

Drug	Stage of development	Targets	Clinical trial identifier
DCC-2701	Phase Ia/Ib in advanced solid tumors	TRKA/B/C , MET, TIE2, and VEGFR	NCT02228811
LOXO-101	Phase Ia/Ib in patients with genetic alterations in TRKA , TRKB , or TRKC	TRKA/B/C	NCT02122913
MGCD516	Phase I/Ib in patients with advanced NSCLC with genetic alterations in MET, AXL, RET, TRK, DDR2, KDR, PDGFRA or KIT, or HNSCC with alterations in MET	TRK , MET, AXL, RET, DDR2, KDR, PDGFRA, and KIT	NCT02219711
PLX7486	Phase I as single agent and in combination with gemcitabine and nab-paclitaxel in solid tumors (pancreatic cancer expansion cohort)	TRKA/B/C and FMS	NCT01804530
RXDX-101	Phase Ia/IIa in patients with genetic alterations in TRKA , TRKB , TRKC , ROS1, and ALK	TRKA/B/C , ALK, and ROS1	NCT02097810
TSR-011	Phase I/IIa in solid tumors and hematologic malignancies (ALK or TRKA positive)	TRKA and ALK	NCT02048488
XL-184	Phase II in advanced NSCLC with NTRK, RET, or ROS1-positive fusions, or increased MET or AXL activity	TRKA , RET, ROS1, MET, and AXL	NCT01639508

NOTE: A chart listing the TRK inhibitors that are currently in clinical trials, additional non-TRK targets, the current stage of clinical development, and the identifier for the relevant clinical trial at clinicaltrials.gov. Items in bold are the primary drug targets. Abbreviations: HNSCC, head and neck squamous cell carcinoma; FMS, McDonough Feline Sarcoma Viral.

of TRK receptors in non-cancer-related diseases is beyond the scope of this review, but can be found elsewhere (86, 87). Interestingly, one of the potential beneficial side effects of targeting TRK receptors in cancer might be a decrease in pain sensation, a frequent symptom among patients with cancer. Conversely, it will be important to monitor for potential neurologic side effects in clinical trials, given the expression pattern of the TRK family of receptors. Currently, little data exist on the toxicities of the more selective TRK inhibitors. A phase I study of PHA-848125AC with significant blood-brain penetration produced dose-limiting (grade 3) ataxia and tremors, but as this drug inhibits cyclin-dependent kinases in addition to TRKA, it is unclear which drug target was responsible for these side effects (88). One of the largest reported studies of a drug with TRKA inhibition was a randomized study of lestaurotinib in *FLT3*-mutant AML (89). Only one neurologic adverse event was noted, a death due to cerebellar toxicity; however, given the multiple kinase targets of this drug, no conclusions can be drawn about the relationship of this toxicity to TRKA inhibition. Monoclonal antibodies against TRKA or NGF have been developed for the treatment of pain (35); however, antibodies to TRKA or other TRK family members would not be effective against TRK fusions, as the extracellular domains for these fusions are routinely lost in the gene rearrangement.

Currently, several TKIs with activity against the TRK family are being explored in clinical trials (Table 2). Two patients with identified *NTRK1* fusions were recently treated with inhibitors that demonstrated the potential clinical benefit of targeting this family of oncogenes. The first was a patient with lung cancer harboring the *MPRIP-NTRK1* fusion, who was treated off-label with the multikinase inhibitor crizotinib (4). Crizotinib has only modest activity against TRKA and produced only a proportionally transient, minor radiographic response,

emphasizing the need for more potent TRK inhibitors in the clinic. More recently, a patient with colorectal cancer with a *TPM3-NTRK1* gene fusion was treated in the phase I portion of a clinical trial investigating an interrupted dosing schedule of RXDX-101 (Ignyta; ref. 90). RXDX-101 is a pan-TRK inhibitor that also has activity against two other gene fusion targets, ALK and ROS1. The TRKA-positive (*TPM3-NTRK1*) patient experienced a partial response, providing the first evidence of clinical activity of a TRK inhibitor in a patient with an oncogenic TRK alteration (90). A phase I/II study of RXDX-101 called STAR-TRK-1 is currently accruing patients with TRK alterations (NCT02097810). LOXO-101 (Loxo Oncology) is a selective pan-TRK inhibitor that has no significant activity outside of the TRK family and is currently being investigated in a phase Ia/Ib trial across multiple tumor types (NCT02122913). TSR-011 (Tesaro) is a TRKA and ALK inhibitor that is currently in a phase I study (NCT02048488). PLX-7486 (Plexxikon) is a pan-TRK inhibitor that also targets FMS (McDonough Feline Sarcoma Viral; CSF1R). It is currently being investigated as monotherapy or in combination with nab-paclitaxel in pancreatic cancer, but there are plans to explore activity in patients with oncogenic TRK alterations in the future (NCT01804530). DCC-701 (Deciphera), XL-184 (Exelixis), and MGCD516 (Mirati) are all multikinase inhibitors that are currently in clinical trials that allow multiple different molecular subtypes, including the TRK family. Lestaurotinib (CEP-701; Cephalon) showed promising preclinical activity in *NTRK1* fusion models, but its clinical development remains unclear (4).

CONCLUSIONS

TRK was one of the first oncogenes identified, more than three decades ago. The TRK oncogenes occur across a broad array of

tumor types. Oncogenic fusions involving *NTRK1*, *NTRK2*, and *NTRK3* and in-frame deletions or splice variants of *NTRK1* are likely to be actionable oncogenes based on preclinical data. The first clinical evidence of tumor response to TRK-targeted therapy in a patient with an *NTRK1* fusion suggests that this family of oncogenes will represent a new valid drug target in cancer.

Disclosure of Potential Conflicts of Interest

A.T. Le has received licensing fees from Abbott Molecular. R.C. Doebele reports receiving a commercial research grant from Mirati Therapeutics; has received honoraria from the speakers' bureau of OxOnc; is a consultant/advisory board member for Loxo Oncology and OxOnc; and has received licensing fees from Abbott Molecular. No potential conflicts of interest were disclosed by the other author.

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